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ASSOCIATION OF XANTHOMONAS PHASEOLI AND THE COMMON BEAN-MOSAIC VIRUS, MARMOR PHASEOLI. II. DISSOCIATION STUDIES OF X. PHASEOLI

FLORENCE HEDGES[1]

(Accepted for publication March 10, 1946)

INTRODUCTION

In earlier studies (4, 5) the writer found that following a long-continued uninterrupted *in vivo* association within stringless Green Refugee variety of common bean (*Phaseolus vulgaris* L.) of the seed-borne infective agents *Xanthomonas phaseoli* (E. F. Sm.) Dowson and bean virus 1 (*Marmor phaseoli* Holmes), the severity of the virus symptoms had greatly increased. Whether this was due wholly or in part to the association of bean virus 1 with the bacterium, to the chance entrance of another virus into the host (5, p. 689), or to the production of a mutant of bean virus 1 was not known. The fact remained that, beginning with the 38th serial passage from bean plant to bean plant of the juice containing the virus and the bacterium, an ultra-severe form of mosaic was produced in 100 per cent of the plants (5, Fig. 1). This persisted from the 38th through the 50th serial passage.

The bacterium, on the contrary, had diminished in its virulence to the point of complete failure to produce symptoms of bacterial infection and to maintain itself in its "typical" virulent S[2] yellow form in the host tissues. This condition persisted for 10 serial passages after which bacterial symptoms re-appeared on the inoculated primary leaves and it was again possible to isolate typical *Xanthomonas phaseoli* from the inoculated bean plants. The 65 checks in this serial passage had no sign of seed infection.

In the interim mildly pathogenic S opaque-white colonies and nonpathogenic S pink ones appeared in the poured plates from the inoculated plants. These aberrant types that had previously been observed from time to time in association with "typical" S yellow colonies were believed to be variants of *Xanthomonas phaseoli.*

In the present comunication the writer reports studies, by means of dissociation experiments, of the changes that the bacterium had undergone during its association with the virus *in vivo.*

MATERIALS AND METHODS

The cultures selected for this study were as follows:

1. A virulent S yellow isolate of *Xanthomonas phaseoli* from the 34th

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[2] Abbreviations are used to designate the type of colony: S, smooth and shining; ± S, more or less smooth and shining; R, rough; ± R, more or less rough; RS, intermediate between rough and smooth or containing both characters (applied to a given culture at a given time); R–S, sometimes rough and sometimes smooth (applied to the white variant of *Xanthomonas phaseoli*).

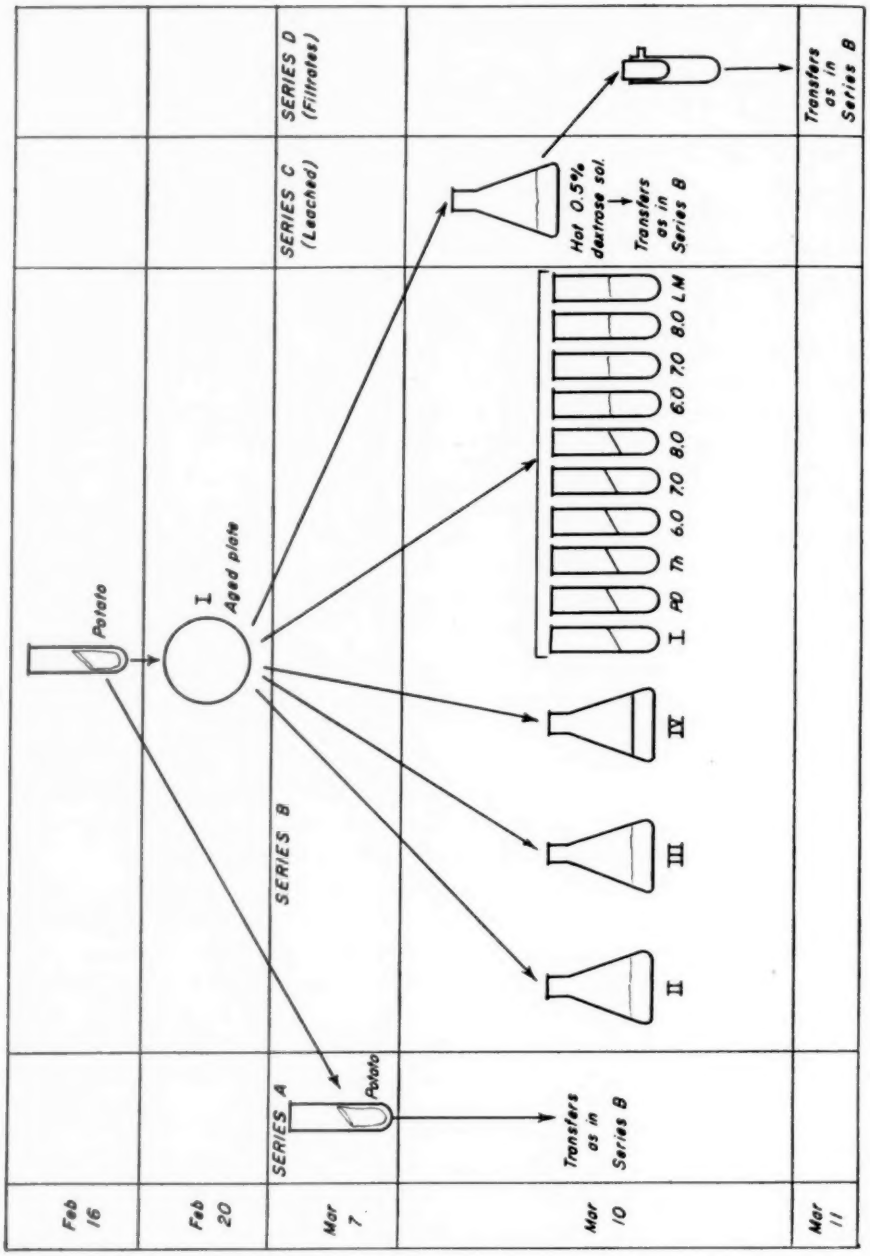

FIG. 1. Diagram of Quirk's dissociation technique used with P(M+P)[21] and "normal" *Xanthomonas phaseoli*. A. Series from young culture as check. B. Series directly from aged, medium-I plate. C. Series from leached contents of aged plate of medium I. D. Series from filtrates of leached contents of aged plate of medium I (filtration apparatus in refrigerator overnight).

Abbreviations: I–IV, Quirk's potato media; PD, potato-dextrose agar; Th, Thaxter's potato-dextrose agar; 6.0, 7.0, 8.0, pH 6.0, pH 7.0, pH 8.0 beef-infusion agars or broths (agar indicated by oblique line in tube); L.M., litmus milk.

serial passage of infected juice from bean plant to bean plant, marking the expiration of two years' uninterrupted association of the common bean mosaic virus and the bacterium *in vivo*. This isolate, designated P(M + P)^{34}, originated from a single colony among only 5 colonies in the isolation plate; and all 5 colonies of the plate resembled those characteristic of the virulent S yellow type-form of *X. phaseoli*. In the designation P(M + P)^{34}, the P denotes *Xanthomonas phaseoli*, the M denotes the mosaic virus, and the superior figure 34 denotes the 34th serial passage of bean juice containing the virus and the bacterium. The 34th serial passage was the second previous to the disappearance of all symptoms of bacterial infection and the fourth preceding the onset of ultra-severe mosaic symptoms.

2. An S yellow, single-colony culture of "normal" *Xanthomonas phaseoli* isolated 8 months before from a lima bean pod and having no history of association with the mosaic virus. This strain likewise was very virulent. It was designated "normal" *Xanthomonas phaseoli*. Like the preceding, it was transferred from a thinly sown plate.

The technique and the media used were those of Quirk (11), to whom the writer is much indebted for suggestions as to their use. The procedure, diagrammatically shown in figure 1, was as follows:

Series A. A check series of each isolate was made from a young culture on steamed potato cylinder.

Direct transfers from 4-day-old steamed-potato-cylinder cultures of the two isolates, P(M + P)^{34} and "normal," were made on February 20 to Petri dishes (150 × 15 mm.) containing a thick layer (8 mm. cir.) of hardened Quirk's medium I. The two strains were indistinguishable in the parent potato cultures. The plate cultures on Quirk's medium I were aged 3 weeks at room temperature. At the end of this period the growth of P(M + P)^{34} was very pale yellow (slightly greenish yellow) with a white margin; the "normal" culture was a deeper greenish yellow with white sectors. These aged cultures were used in Series B, C, and D.

Series B. Transfers of the 2 isolates were made directly from the aged plates to flasks filled to a depth of 1 to 1½ inches with Quirk's media II, III, and IV and to tubes of Quirk's medium I, Potato-dextrose agar, Thaxter's potato-dextrose agar, Beef-infusion agar at pH 6.0, pH 7.0, and pH 8.0, Beef-infusion broth at pH 6.0, pH 7.0, and pH 8.0, and Litmus milk. In other words, the tubes comprised a set of the culture media most commonly used for phytopathogenic bacteria plus Quirk's medium I. Later, steamed potato cylinders, upon which "typical" *Xanthomonas phaseoli* grows very characteristically,[3] were included in the tube setup. No beef-extract agars were used.

Series C (leached). In this paper the term "leaching" is applied to a soaking or steeping of material from the aged plate cultures. The proce-

[3] Copious, smooth, shining, more or less fluid or "sirupy" growth of varying shades of yellow. If potato cylinders half covered with water are used before they have been allowed to dry out at all, the growth usually so fills the liquid that the cultures can be turned upside down with impunity.

dure was as follows: Fifty cc. of 0.5 per cent dextrose solution was autoclaved in 500-cc. flasks and cooled to 60° C. cir. At this point were transferred to the flasks small pieces of the agar substratum from the aged plates after the greater part of the bacterial growth had been removed with a sterile swab and discarded. About ⅓ of the layer of agar in the Petri dish was used. The dextrose solution was hot enough to soften the agar and expedite the freeing of any filterable forms that might have penetrated it, and at the same time the solution cooled off too quickly to kill *Xanthomonas phaseoli,* the thermal death point of which is about 50° C. with a 10-minute exposure. The flasks were well shaken and allowed to stand about 20 minutes. Transfers from the leach were then made to the same media as in Series B.

Series D. The suspensions of the two isolates in the 0.5 per cent dextrose leaching solution used in Series C were filtered by gravity through a Chamberland-Pasteur L 3 filter immediately after making the Series C transfers. As this step was reached so late in the afternoon that insufficient filtrate was obtained for inoculum by nightfall, the filtration was completed in a refrigerator held at 10° to 12° C. and transfers like those in the preceding series A, B, and C, were made early the next morning.

Quirk's media I–IV. The following directions for making the special potato media used in these investigations were furnished by Miss Quirk.

Medium I, yeast-potato-mannitol-nitrate agar with oxidized potato-extract base, prepared as follows: Scrub potatoes thoroughly, pare, grind in a meat grinder. To 2 parts distilled water add one part potato. Allow to stand in open glass or enamel receptacle in refrigerator overnight. Next morning stir mixture well to distribute starch. Filter through cheesecloth to let juice and some of the starch pass. Put oxidized juice and starch into large flasks and autoclave one hour at 115° C. Let stand overnight in flasks to precipitate the cooked starch. Decant the supernatant liquid and filter through cotton. Do not add water to make up to volume.

To 500 cc. oxidized potato extract, add 100 cc. yeast solution,[4] 500 cc. distilled water, and 15 g. agar. Cook mixture for one hour in steamer. Filter through cotton, add the following chemicals: 10.0 grams Mannitol, 0.2 g. NaCl, 0.2 g. K_2HPO_4, 0.2 g. $MgSO_4$, 0.1 g. $CaSO_4$, 1.0 g. $CaCO_3$, and 1.0 g. KNO_3.

Stir chemicals well into hot agar (steam 10 or 15 minutes if necessary). Without filtering mixture, distribute medium in small flasks to a depth of 1 to 1½ inches. Autoclave 20 minutes at 115° C.

Medium II, unoxidized-potato agar (4 per cent). Prepared as Thaxter's potato-dextrose agar (2, p. 154) but with an additional 2½ per cent agar. Slice potatoes thin and immediately cover with distilled water to prevent oxidation. To 2 parts of distilled water add 1 part potato. Steam 20 minutes, or let simmer in a water-bath, below 60° C., for 1 hour. Filter

[4] Stock yeast solution: 100 g. yeast cakes (Fleishman's), 1000 cc. of distilled water. Cook 4 hours in steamer, stir occasionally, allow yeast to settle. Decant supernatant liquid and autoclave 20 minutes at 115° C.

through cheesecloth and make up to original amount with distilled water. Add 2 per cent dextrose and 4 per cent agar. Cook 1 hour. Filter through cotton. Distribute in small flasks. Autoclave 20 minutes at 115° C.

Medium III, oxidized-potato-extract agar (4 per cent). Prepare potato extract and yeast solution as for medium I. Add 2 per cent of dextrose and 4 per cent of agar. Cook 1 hour. Filter through cotton. Distribute in small flasks to a depth of 1 to 1½ inches. Autoclave 20 minutes at 115° C.

Medium IV, potato-starch medium. Precipitated sterile starch jelly obtained while making oxidized potato extract for media I and III. This cooked precipitated starch is washed well under running tap water. The washed starch jelly is then distributed in small flasks to a depth of 1 to 1½ inches. Autoclave 20 minutes at 115° C. (No ingredients added to the starch jelly in medium IV.)

Pathogenicity testing. The pathogenicity of the dissociated cultures was tested by rub-inoculations on the underside of the primary leaves of young Stringless Green Refugee bean seedlings. Both the undiluted bacterial growth and sterile-distilled-water suspensions of the same were used as inoculum. A large number of noninoculated isolated checks was always held.

The line of Stringless Green Refugee bean used in the pathogenicity tests was the same as that used in the serial passages described in the earlier paper (5, p. 666). The seed was collected by W. J. Zaumeyer from healthy plants in his breeding plots at Greeley, Colorado.

CULTURAL STUDIES

I. *Dissociated Cultures Prior to Filtering*

By the Quirk technique of demonstrating dissociation, most striking differences between the P(M + P)[34] strain and "normal" *Xanthomonas phaseoli* were readily revealed. This showed to be a fact that which the writer had suspected from the behavior of the bacterium in the serial passages, namely that *X. phaseoli* had undergone considerable change during its two years' uninterrupted association with the virus *in vivo*.

Strain P(M + P)[34]. Transfers from the aged plate before and after leaching (Fig. 1, Series B and C, respectively). The bacterial isolate which had been associated continuously for two years with the virus *in vivo* was readily dissociated into:

1. A dominant white form, varying from R to S and only mildly pathogenic.
2. A subordinate S yellow form, the "typical" virulent form.
3. An S pink form.[5]

On all solid media, including those in the tube series (Fig. 2, A and C), inoculated either before or after leaching, the ± R white variant appeared

[5] This is believed to be the pink form encountered from time to time, usually as a minority group, in plates from bean plants inoculated with the two associated infective agents. If so, it is nonpathogenic. In the dissociated cultures under discussion, it always appeared in conjunction with either the yellow or white forms.

first and was very much to the fore. Relatively little of the virulent S yellow type developed in transfers made either before or after leaching but its best development was on Quirk's medium IV, inoculated before leaching. There was only a trace of it in the tube transfers, namely on medium I, potato-dextrose agar, and Thaxter's agar made before leaching, and only on the two last mentioned made after leaching.

No S pink form appeared in transfers made prior to leaching with the hot dextrose solution. After leaching, this form reached its greatest development in transfers to flasks of Quirk's media II and III. In the tube series it appeared in small quantities on Quirk's medium I, potato-dextrose agar, and Thaxter's agar.

All three color types showed up most strikingly on Quirk's medium II inoculated after leaching. The first to appear (in 18 hours) was an S chalk-white growth which gradually changed to RS and spread over the surface, the only type visible during the first 5 days. The 7th day this white layer was dotted with numerous small, raised S yellow and S pink colonies.

During the 34 serial passages covering a 2-years' association with the virus *in vivo*, some factor or factors had encouraged the development of the less infectious R–S opaque-white and the nonpathogenic S pink variants at the expense of the virulent S yellow type commonly recognized as *Xanthomonas phaseoli*. This "typical" virulent form was assuming a subordinate rôle and having difficulty in asserting itself.

"Plaques,"[6] appeared in all beef-infusion agars inoculated before and after leaching, on potato-dextrose agar before leaching, and on Quirk's medium I after leaching.

Second lineal transfers from the aged plate of $P(M+P)^{34}$. Transfers were made to potato-dextrose agar, Thaxter's potato-dextrose agar, and pH 7.0 beef-infusion agar from each of the flasks of Quirk's media II, III, and IV, when the latter were 13 days old.

The $P(M+P)^{34}$ strain of *Xanthomonas phaseoli* still dissociated in these second lineal transfers from the aged plates.

The mildly pathogenic R–S white variant was the first to appear in all cultures of the nonleached series. The white form likewise developed first in the leached series with but two exceptions (on potato-dextrose and on Thaxter's agars, in transfers from Quirk's medium III) where only the "typical" S yellow form and a trace of S pink appeared. The R–S white variant was the only type to appear on potato-dextrose agar from Quirk's medium III in the nonleached series or on pH 7.0 agar from any source.

The virulent S yellow form, nevertheless, was beginning, in these second lineal transfers, to recover from the subordination so marked in the initial cultures made from the aged plate (Fig. 1, Series B and C). It was regaining its normal dominance to a greater extent in the leached than in the nonleached series.

[6] Small clear areas resembling the plaques (bare spots) described by d'Herelle (7, p. 12, 20, and 95) as due to the lytic action of bacteriophage.

The S pink form was present in the leached set only. It appeared in cultures on potato-dextrose agar and on Thaxter's agar from all sources.

All three types appeared together in the potato-dextrose and the Thaxter's agars from Quirk's media II and IV in the leached series.

"Normal" strain. Tranfers from the aged plate before and after leaching (Fig. 1, Series B and C, respectively). The "normal" *Xanthomonas phaseoli* was much less readily dissociated than was P(M + P)[34]. The preliminary ± R white growth of the R–S white variant, so prominent in P(M + P)[34], appeared only in transfers made after leaching with the hot 0.5 per cent dextrose solutions though as the culture grew older some S white appeared on medium IV in the nonleached set. The R–S white form was conspicuous only in the flask cultures of Quirk's media II, III, and IV on which the dissociation phenomena are far better demonstrated than in the media most commonly used for bacterial plant pathogens. On Quirk's media II, III, and IV in the leached series there was an S white growth in 18 hours which was, however, quickly overrun by the "typical" virulent S yellow type so that in 5 days but little of the white growth was visible in any of the flasks. Ultimately it was covered completely. It was noted that the R–S white variant in this leached set of "normal" *X. phaseoli,* though overrun so quickly by the S yellow type, was apparently less subjugated by the latter on medium II than on media III or IV, as was shown by second lineal transfers.

In the tube cultures transferred either before or after leaching from the aged plates of Quirk's medium I, *viz.,* to medium I, potato-dextrose agar, Thaxter's potato-dextrose agar, pH 6.0, pH 7.0, and pH 8.0 beef-infusion agars, there was a striking contrast between the S yellow growth of this "normal" *Xanthomonas phaseoli*[7] and the ± R white growth of P(M + P)[34] (Fig. 2). Another notable difference between the two strains on these commonly used media was the much greater inhibiting effect of leaching on the "normal" *X. phaseoli.*

The S pink form appeared in "normal" *Xanthomonas phaseoli* only after filtering, a further contrast to P(M + P)[34]. This was also true of second lineal transfers.

"Plaques" appeared less frequently than in P(M + P)[34] and then only after leaching, *viz.,* on pH 6.0 and pH 7.0 beef-infusion agars.

Second lineal transfers from the aged plate of "normal" Xanthomonas phaseoli. Transfers were made from each of the flasks of Quirk's media II, III, and IV (when 13 days old) to potato-dextrose agar, Thaxter's potato-dextrose agar, and pH 7.0 beef-infusion agar.

Unlike P(M + P)[34], the "normal" *Xanthomonas phaseoli,* with a few outstanding exceptions, dissociated but little in these second lineal transfers from the aged plate. The R–S white variant failed to appear in the majority of transfers (both nonleached and leached sets) and occurred only as a trace in a few others. The few outstanding exceptions to this minor

[7] There was some slight out-cropping of white on most solids at one time or another in the tube set of "normal" *X. phaseoli* made after leaching.

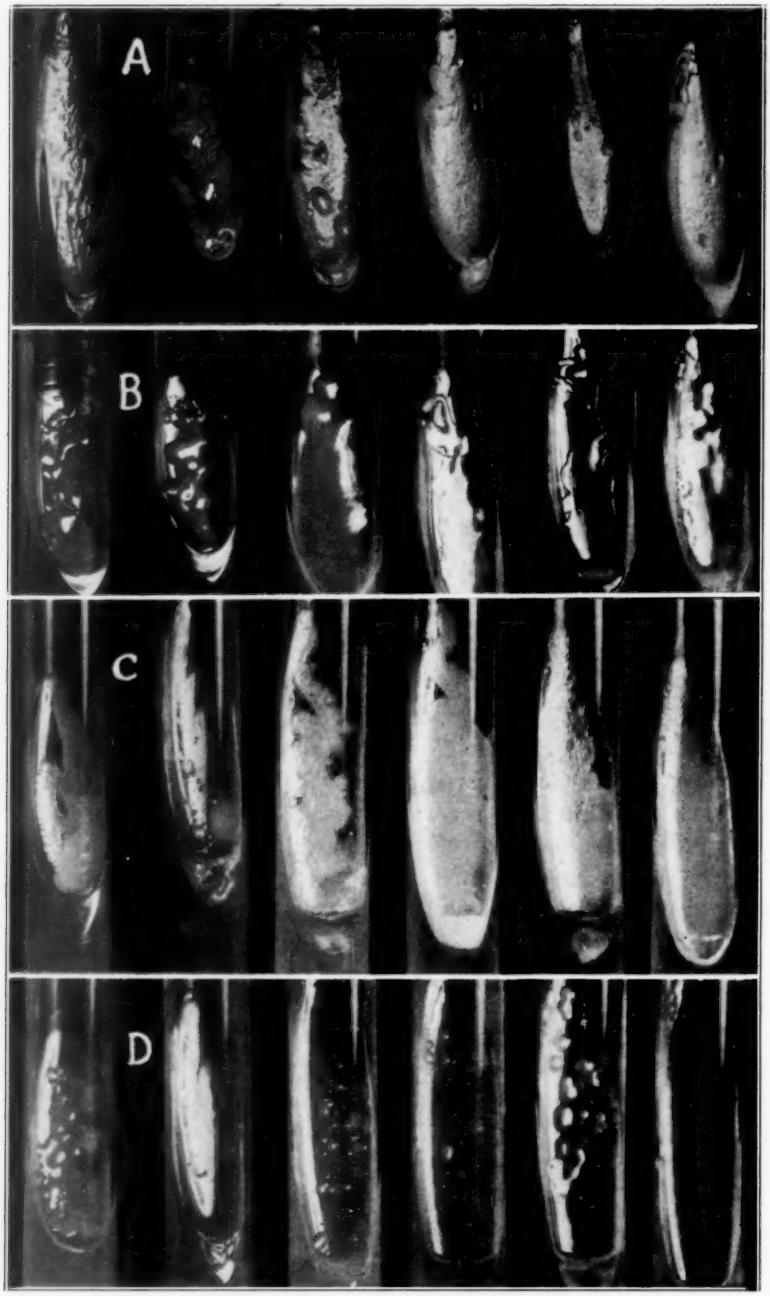

FIG. 2. Eleven-day-old transfers from aged plates of Quirk's medium I before and after leaching, showing difference between P(M + P)[34] (*X. phaseoli* associated continuously *in vivo* for 2 years with bean virus 1) and "normal" *X. phaseoli*.

Media, left to right: Quirk's I agar, potato-dextrose agar, Thaxter's potato-dextrose agar, pH 6.0, pH 7.0, pH 8.0 beef-infusion agar. A. P(M + P)[34] cultured before leaching showing good ± R white growth; trace of S yellow type-form in some. B. "Normal" strain cultured before leaching; good "typical" S yellow growth characteristic of the virulent type-form of *X. phaseoli*. C. P(M + P)[34] cultured after leaching; inhibition on potato-dextrose agar; good ± R white growth on all others, trace of S yellow and S pink forms. D. "Normal" strain cultured after leaching; marked inhibition of S yellow growth; trace of whitish growth in some.

rôle of the mildly pathogenic R–S white form were on pH 7.0 beef-infusion agar from medium II in the nonleached set and the transfers to all three media from medium II in the leached set. In all four of these cultures, the R–S white form was dominant and, furthermore, it was the only one to appear in two of them. The dominance of the R–S white form in all three transfers from medium II leached was all the more noteworthy because at the time these transfers were made, the preliminary ± R white growth in the parent flask culture had been completely overrun by the virulent S yellow type-form.

The virulent S yellow form was dominant in all other second lineal transfers, occurring in seemingly pure culture in the majority and growing as hitherto considered characteristic[8] of *Xanthomonas phaseoli*. There was no trace of the S pink variant.

II. *Behavior of Check Transfers from Young Cultures*

At the same time that transfers of the two isolates, P(M + P)[34] and "normal" *Xanthomonas phaseoli*, were made from aged plates of medium I to flasks of Quirk's media II, III, and IV and to the tube setup of Quirk's medium I and the media most commonly used for bacterial plant pathogens, a parallel set of media was inoculated from young (3-day-old) steamed-potato cultures (Fig. 1, Series A). Both the undiluted bacterial growth and a suspension in pH 7.0 beef-infusion broth were used as inoculum. There was no leaching.

Both P(M + P)[34] and "normal" *Xanthomonas phaseoli* from young cultures produced, in 18 hours, the good S yellow growth characteristic of the virulent form of this organism. There was no trace of the preliminary chalk-white growth varying from R to S which was dominant in all P(M + P)[34] transfers from aged plates of Quirk's medium I and which, in the case of "normal" *X. phaseoli*, was visible for a short time only in flask transfers made to Quirk's media II, III, and IV from the aged plates after leaching.

As the transfers from young parent cultures grew older, some traces of a white form appeared in some of them as whitish streaks or patches, and on medium IV an infiltrating white form appeared. This was true of both "normal" *Xanthomonas phaseoli* and P(M + P)[34]. No S pink form appeared in either strain.

In short, when young cultures were used as inoculum for a comparison of the two strains, the inherent differences between them were not evident on either the media most commonly used for bacterial plant pathogens or on Quirk's media I to IV.

III. *Filtrates of Dissociated Cultures*

Very much less difference between the strains P(M + P)[34] and "normal" *Xanthomonas phaseoli* with regard to dissociation was apparent in cultures

[8] Copious syrupy S yellow growth on media rich in carbohydrates.

from the filtrates (Fig. 1, Series D) than was found between them when cultured after aging and leaching, but prior to filtering. In each strain a white or colorless form varying from R to S and, for the most part, but mildly infectious came through the filter. In some cases there was also an S yellow form.

In the second or third lineal transfers from the filter tubes (both strains), the S pink variant not infrequently put in an appearance. The pink form occurred in the form of superimposed colonies or as streaks or sectors in the white or greenish yellow S growth. The S pink variant was much more conspicuous in the P(M + P)[34] strain than in the "normal" strain.

Of special interest are the facts that:

1. The filtrate of neither strain produced growth in more than one of the commonly used culture media in the tube setup made directly from the filter tubes as soon as possible after filtering (Fig. 1, Series D). There was growth in pH 7.0 beef-infusion broth with P(M + P)[34] and in pH 6.0 broth with "normal" *Xanthomonas phaseoli*. On the other hand each strain produced growth of some kind on Quirk's media II, III, and IV in the flask setup of the same date.

2. The barely visible, finely roughened, film-like growth occurring on Quirk's medium II with P(M + P)[34] and on Quirk's media II and III with the "normal" strain was wholly unlike anything previously encountered with *Xanthomonas phaseoli*. Smears from the films on medium II (both strains) showed the presence of rods and cocci. Filaments also were present in the "normal" strain smears, some very long and branched, some segmented and breaking up. Such pleomorphism is characteristic of R forms in general and has been reported for many animal pathogens. Transfers from the P(M + P)[34] film produced clouding in pH 7.0 beef-infusion broth and an S greenish yellow growth with white margin on pH 7.0 beef-infusion agar. A pH 7.0 agar subculture of the last mentioned had striking S pink sectors in the S greenish yellow nailhead. No visible growth appeared in transfers from the P(M + P)[34] film to potato-dextrose agar or Thaxter's potato-dextrose agar.

Transfers from the "normal" strain film on Quirk's medium II produced an R colorless film on pH 8.0 beef-infusion agar but no visible growth on any other of the media most commonly used for phytopathogenic bacteria.

The film produced by the "normal" strain on Quirk's medium III was similar in appearance to those above mentioned but was interspersed with clear areas and consisted, at the time of examination, of spherical bodies (which may have been filaments broken up). It resembled ice thawing and breaking up on a lake. Transfers from this to a set of the most commonly used culture media produced an RS chalk-white growth on pH 6.0 beef-infusion agar, an R colorless film on pH 8.0 agar, and a scant growth of S colorless colonies on Quirk's medium I. There was no visible growth on other media.

3. The filtrate of the "normal" strain did not revert to the "typical"

S yellow form *in vitro* through aging in pH 6.0, pH 7.0, or pH 8.0 beef-infusion broths. The term "reversion" is used in this paper in the sense of a return to the type-form of *Xanthomonas phaseoli*. This might result (a) if individual cells in the "atypical" growth gave rise to progeny containing both "atypical" and "typical" forms of *Xanthomonas phaseoli* and the latter subsequently gained predominance or (b) because of the development of the "typical" form already present but masked in the "atypical" growth or from a combination of (a) and (b).

The transfers to the pH 6.0, pH 7.0, and pH 8.0 beef-infusion broths for aging were made on April 25 from the March 11 culture on medium IV when it was $6\frac{1}{2}$ weeks old (see Fig. 6). At intervals up to the 76th day, transfers to a tube setup were made from these broth cultures with the following results:

Not even a trace of a yellow form came up in any transfer from the filamentous form of the R–S white variant in the pH 6.0 broth. Greenish yellow growth appeared in early transfers from pH 7.0 and 8.0 broths but by the 76th day the R–S white variant was dominant in subcultures of each. There was a trace of the S pink form in some transfers from each of the three broths. In other words there was at no time, in any of the subcultures of these broth transfers taken from the aged March 11 flask culture on medium IV, any growth resembling that of the "typical" virulent S yellow form of *Xanthomonas phaseoli*.

On the other hand, in 10 days there was reversion *in vivo* of the R–S white variant from the "normal" strain filtrate to "typical" S yellow *Xanthomonas phaseoli*. The inoculum was from a pH 7.0 beef-infusion agar transfer from the same March 11 flask culture on medium IV, but made when the latter was but 12 days old (Fig. 6, Inoc. 3).

4. After 10 days' incubation of the filtrate in the 0.5 per cent dextrose solution in the filter tubes,[9] transfers to Thaxter's agar from both strains gave the characteristic S yellow growth of "typical" *Xanthomonas phaseoli*. In the P(M + P)[34] strain there were white patches of the R–S white variant as well.

BEAN PLANT INOCULATIONS

I. *Dissociated Cultures Used as Inoculum Prior to Filtering*

A. *Flask cultures on Quirk's medium IV as inoculum.*

Two-week-old cultures on Quirk's medium IV made from the aged plates of Quirk's medium I before and after leaching were used as inoculum on Stringless Green Refugee bean seedlings.

Results with the "normal" strain (Fig. 4, March 24 inoculations 1 and II). The "normal" *Xanthomonas phaseoli*, both before and after leaching, was extremely virulent, markedly more so than was P(M + P)[34]. It worked more rapidly and caused a much more severe type of bacterial infection. The difference was particularly marked in the secondary infection on the

[9] After the initial transfers, the tubes of filtrate had been kept under observation. In 10 days the "normal" filtrate was still clear, that of P(M + P)[34] clouded.

stems where, with the "normal" strain, there was much external discoloration in 28 out of 29 (96½ per cent) of the plants inoculated March 24; and 22 of them had bacterial ooze. The pathogenicity of the "normal" strain was little affected by the leaching, probably because the less virulent R–S white variant which came up in the young cultures was so quickly overrun by the S yellow type-form of *X. phaseoli*.

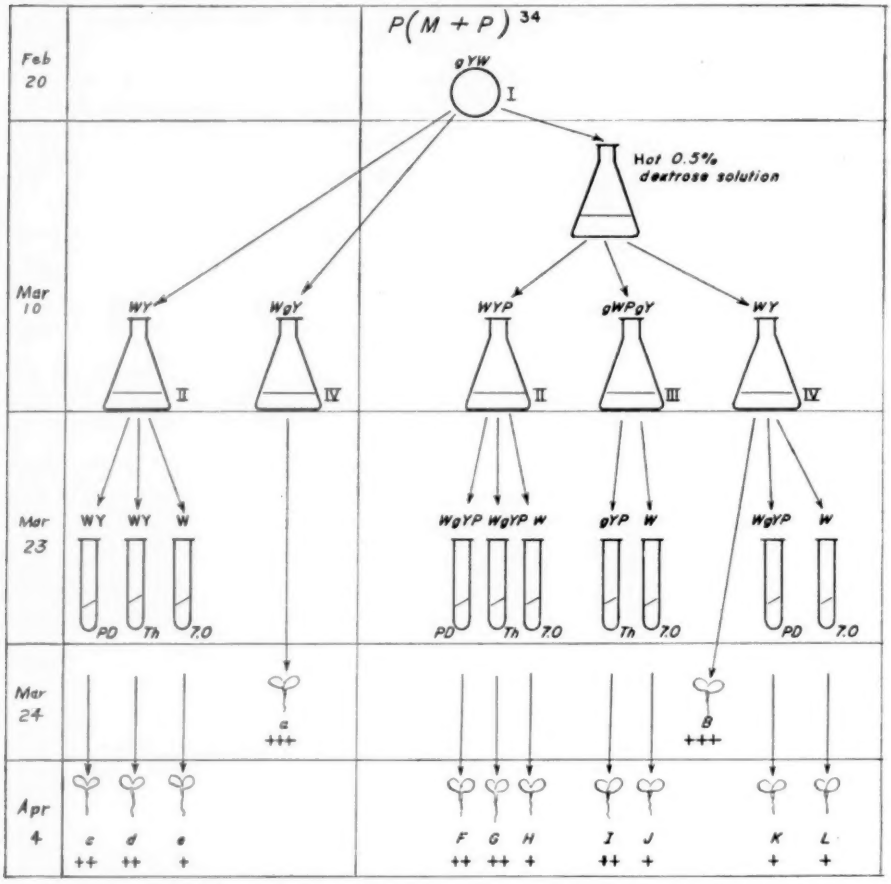

FIG. 3. Inoculations of bean plants with dissociated P(M + P)[34] before and after leaching, showing ancestry and color of cultures used as inoculum. Plus signs are qualitative rather than quantitative symbols of bacterial infection.

Abbreviations: Media: I–IV, Quirk's potato media; PD, potato-dextrose agar; Th, Thaxter's potato-dextrose agar; 7.0, pH 7.0 beef-infusion agar. *Color of growth:* P, pink; W, white; Y, yellow; w, trace white; y, trace yellow; gW, greenish-white; gY, greenish-yellow.

This greater virulence of the "normal" strain was to be expected since the dissociation of the two strains had shown that the "normal" contained a much greater concentration of the highly virulent S yellow form than did P(M + P)[34]. No seed infection was observed in the checks.

Results with P(M + P)[34] (Fig. 3, March 24 inoculations a and B). Cultures of P(M + P)[34] on Quirk's medium IV produced, like those of the nor-

mal strain, 100 per cent bacterial infection, both before and after leaching, but though this was good to excellent on the inoculated primary leaves, the secondary infection on the stems was very mild. There was also evident in this strain, a marked lessening of the invasion of stem tissues in the plants inoculated with cultures in the leached series. This was not surprising inasmuch as a study of the dissociated cultures had shown that the leaching

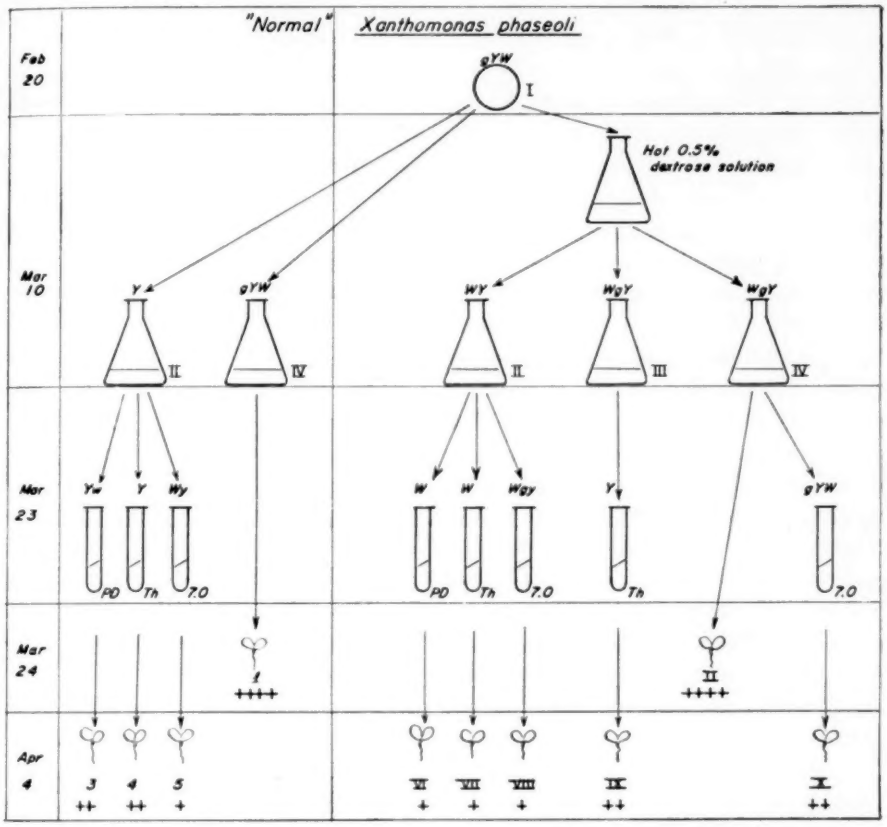

FIG. 4. Inoculations of bean plants with dissociated "normal" *Xanthomonas phaseoli*. All data parallel those in figure 3.

had further favored the weakly-pathogenic or avirulent variants, R–S white and S pink, seemingly already encouraged by the 2 years' association with the virus *in vivo*. The majority of the plants inoculated with $P(M+P)^{34}$ before leaching showed slight but distinct stem discoloration and some internal browning. On the contrary, of those inoculated with leached $P(M+P)^{34}$, 14 out of 22 (64 per cent) showed no external or internal sign of stem infection though microscopic examination revealed the presence of bacteria in the stem tissues of 21 of the 22. Bacterial ooze was present in but 4 of the 33 plants inoculated March 24, 2 out of 11 in the nonleached series, and 2 out of 22 in the leached series. No seed infection was observed in the checks.

B. *Subcultures from initial flask cultures as inoculum (Second lineal transfers from aged plates of strain P(M + P)[34] and "normal" strain).*

No direct inoculations were made from flask cultures on Quirk's media II and III from the aged plates of the two strains before and after leaching, but 15 selections from 12-day-old transfers from said flask cultures (when two weeks old) to potato-dextrose, Thaxter's potato-dextrose, and pH 7.0 beef-infusion agar (Figs. 3 and 4, March 23 cultures) were tested for pathogenicity on 2-week-old Stringless Green Refugee bean seedlings.

On the same date, 12-day-old transfers from flask cultures on medium IV from the aged plates (both strains; leached series) were tested on bean plants for comparison with the March 24 inoculations with the parent cultures on medium IV *per se* recorded above (Section A).

Results of inoculation (both strains, Figs. 3 and 4, April 4 inoculations). The difference in results obtained with the two strains was on the whole much less marked in these inoculations with the second lineal transfers than in those described in Section A made with the initial flask cultures on medium IV transferred directly from the aged plates (Figs. 3 and 4, March 24 inoculations). All the second lineal transfers from the aged plates of each strain produced bacterial infection, although in some sets there was no external sign of primary infection on the rubbed leaves or of secondary infection on the stems (both strains); the plants in such cases were believed to be healthy until microscopic examination revealed the presence of bacteria in the stems. Such masked infection occurred in 4 sets of inoculations with the "normal" strain (Fig. 4, April 4 inoculations 5, VI–VIII) and in 2 of the P(M + P)[34] sets (Fig. 3, April 4 inoculations e and J). It ranged from $16\frac{2}{3}$ to 60 per cent of the plants inoculated with "normal" and from 35 to $66\frac{2}{3}$ per cent with P(M + P)[34]. (The plus signs in figures 3 and 4 are qualitative rather than quantitative symbols.)

Though the 18 transfers were pathogenic in varying degrees (both strains) none was sufficiently virulent to cause severe leaf infection, premature killing of primary leaves, or girdling, splitting, or breaking of the stems. Where the R–S white variant was dominant in either strain (more often than not on pH 7.0 beef-infusion agar), there was mild or poor, usually masked, infection. Sometimes the signs of disease were confined to barely perceptible discoloration of a single vessel in the stem. There was always more or less good infection with either strain wherever there was a fair amount of the S yellow form in the culture used as inoculum.

There was no obvious effect of the S pink form on the virulence of the S yellow form.

The subcultures from Quirk's medium IV leached (both strains) were much less virulent (Figs. 3 and 4, April 4 inoculations K, L, and X) than had been those from the parent flasks *per se* (Figs. 3 and 4, March 24 inoculations B, II). This decrease in virulence in the second lineal transfers was more pronounced with P(M + P)[34]. The subcultures from medium IV leached (both strains) had been made the day preceding the use of the

2-week-old parent flasks as inoculum on March 24, and were themselves used for inoculation at approximately the same age.

No evidence of seed-borne infection (bacterial or virus) was observed in the large number of noninoculated checks nor did microscopic examination of these healthy appearing plants reveal the presence of bacteria.

II. *Filtrates of Dissociated Cultures Used as Inoculum*

With the filtrate of each strain grown on various media, bacterial infection, mild for the most part, was produced on Stringless Green Refugee bean seedlings (Figs. 5 and 6). No culture was wholly avirulent.

With a single exception, no visible leaf infection was produced with the filtrate of either strain of the organism when the inoculum consisted of (a) any of the initial transfers of March 11, (b) subcultures of the same, or (c) the contents of the filter tube *per se* after 10 days' incubation.

In the exceptional case cited (Fig. 6, April 4 inoculation 3 with pH 7.0 beef-infusion agar subculture of the "normal" strain on medium IV) the R–S white variant produced inconspicuous water-soaking on the rubbed primary leaves and mild stem infection in 40 per cent of the plants. Isolation plates from the watersoaked spots on one of the rubbed leaves 10 days after inoculation, showed that the R–S white variant had, for the most part reverted in the host to "typical" S yellow *Xanthomonas phaseoli*. There were innumerable S yellow colonies and but 4 colonies of the R–S white form. At the time it was used for inoculum, the R–S white variant had been making a ± S growth and the 4 colonies mentioned were S white.

Stem infection was produced by all the filtrate cultures although in some cases (both strains) there were little or no external signs of this. However, microscopical examination revealed bacteria in 9 to 100 per cent of these seemingly healthy plants. (Plus signs in figures 5 and 6 are qualitative rather than quantitative symbols.)

The only severe infection on both leaves and stems (Figs. 5 and 6, May 16, inoculations g and 9) was produced by potato cultures (both strains) descended via Thaxter's potato-dextrose agar, from the bacterial suspension in the filter tubes[10] incubated 10 days, during which time the "typical" virulent S yellow form of *Xanthomonas phaseoli* had developed. There was 100 per cent excellent infection on the rubbed primary leaves, with girdling, splitting, and breaking over of the stems accompanied by extrusion of bacterial slime. It is interesting to note by way of contrast that inoculations made directly from the bacterial suspension in the 10-day-old filter tube (both strains) had produced no visible infection on the leaves and only mild symptoms on the stems although 100 per cent of the plants inoculated with P(M + P)[34] (filter tube clouded) and 40 per cent of these inoculated with "normal" *X. phaseoli* (filter tube still clear) were infected (Figs. 5 and 6, March 20 inoculations a and 1).

Plates poured from one of the severely infected stems from the

[10] The identical filter tubes had been used for the initial transfers.

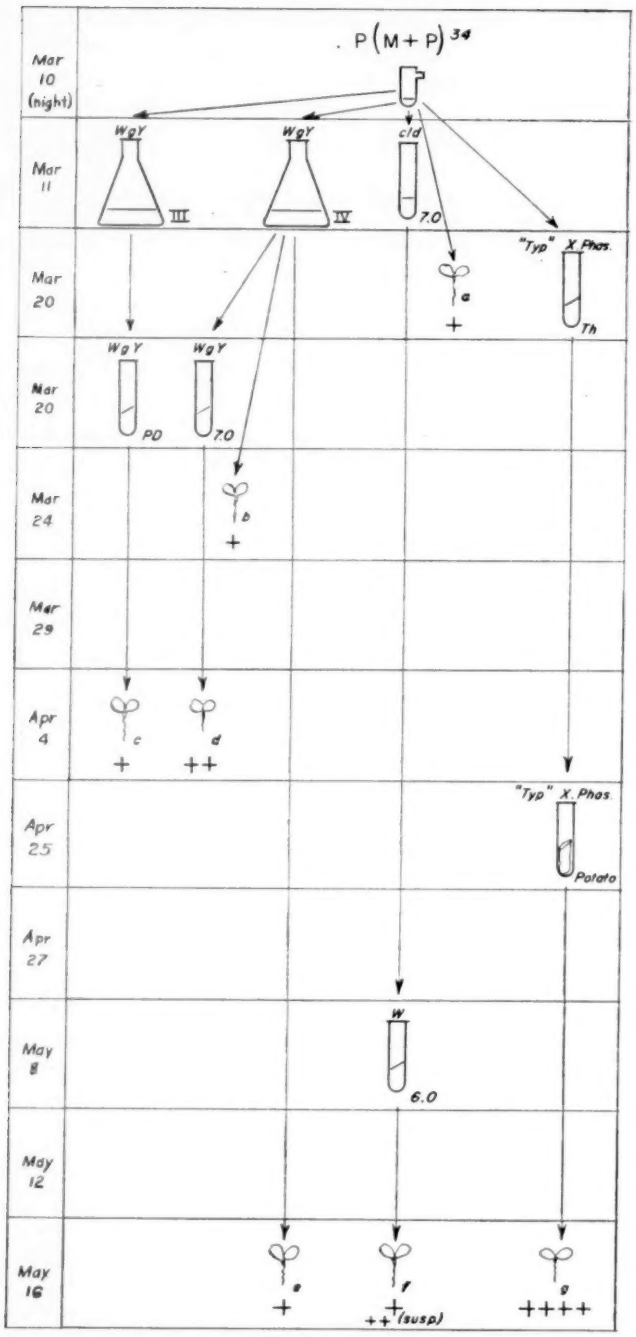

FIG. 5. Inoculations of bean plants with filtrates of leached plate culture of P(M+P)[34] aged on Quirk's medium I, showing ancestry and color of cultures used as inoculum. Plus signs are qualitative rather than quantitative symbols of bacterial infection.

Abbreviations: Media: III and IV, Quirk's potato media; PD, potato-dextrose agar; Th, Thaxter's potato-dextrose agar; 6.0, 7.0, 8.0, pH 6.0, pH 7.0, pH 8.0 beef-infusion agars or broths; Susp., sterile-distilled-water suspension of bacteria. *Growth:* Cld., clouded; fil., filaments; W, white; Y, yellow; pW, pinkish white; gY, greenish yellow; y, trace yellow.

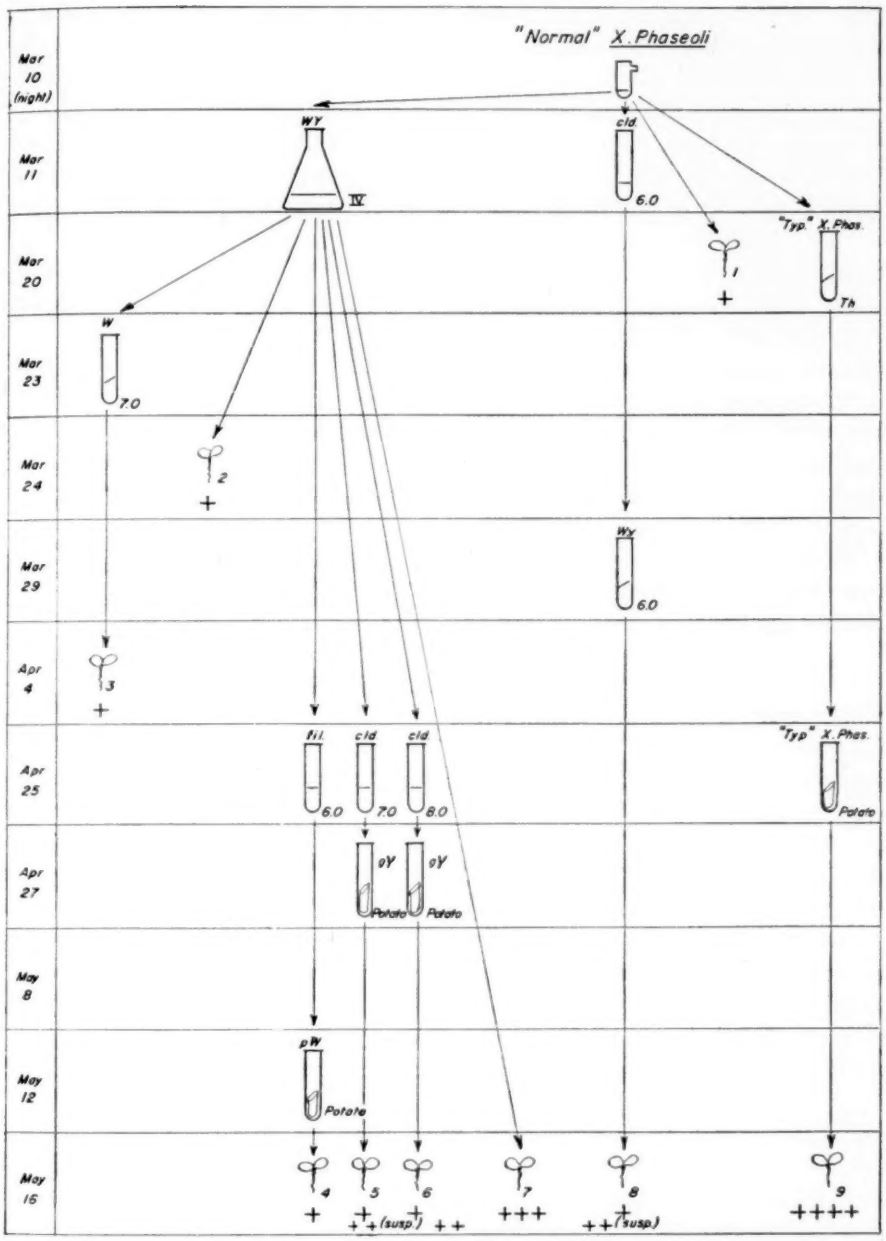

FIG. 6. Inoculations of bean plants with filtrates of leached plate culture of "normal" *Xanthomonas phaseoli* aged on Quirk's medium I. Reversion of R–S white form to "typical" S yellow *X. phaseoli* occurred in inoculation No. 3; April 4. All data parallel those of figure 5.

P(M+P)^{34} inoculation set g, contained both "typical" *Xanthomonas phaseoli* and the mildly pathogenic yellow variant of *X. phaseoli* described by the writer in an earlier paper (5, p. 665 and footnote 12). The variant was much in the majority.

Mosaic was produced in 100 per cent of the plants inoculated with nondiluted bacterial growth from the aged (2 months) initial flask culture on Quirk's medium IV of the filtrate of P(M+P)^{34} (Fig. 5, May 16 inoculation e). There were no macroscopic signs of bacterial infection. Unfortunately only 6 plants were included in this set. The 11 plants inoculated at the same time with a suspension of bacteria from the same flask culture showed no symptoms of either mosaic or bacterial infection but microscopical examination revealed masked bacterial infection in 36⅓ per cent of them.

In the comparable inoculation of 15 plants with the "normal" strain filtrate (Fig. 6, May 16 inoculation 7) there was 100 per cent severe bacterial infection causing breaking over and killing of stems but no visible leaf lesions. Among the 11 plants inoculated with the bacterial suspension, 4 showed mosaic symptoms.

In the large number of noninoculated check plants no sign of seed-borne infection, bacterial or virus, was observed nor were masked bacteria revealed by microscopical examination of these healthy-appearing plants.

DISCUSSION

The P(M+P)^{34} culture used for the dissociation studies originated from a single colony of *Xanthomonas phaseoli*. It was transferred from an isolation plate containing but 5 colonies and these well distributed. The "normal" strain likewise originated from a single colony in a thinly-sown plate. The colonies of the 2 strains were indistinguishable in appearance as were also the young cultures on steamed potato cylinders used as source inoculum for the dissociation experiments.

The conditions prevailing in the much stunted bean plants of the 34th serial passage of the 2 infective agents from which P(M+P)^{34} was isolated were such as those described by Riker (12) as being particularly conducive to variation in bacterial plant pathogens, namely those "preventing rapid vegetative growth."

That variants existed in the serial passages had previously been shown by isolation plates as reported in an earlier paper (5). It was presumable, therefore, that the bacterial population in these dwarfed plants of the 34th serial passage of the associated infective agents was a complex of variants of *Xanthomonas phaseoli* and that, furthermore, these might be masked in colonies outwardly indistinguishable from those of "normal" *X. phaseoli*.

The object of these studies was to determine whether a colony transfer of P(M+P)^{34} when subjected to a dissociation technique would differ from one of "normal" *Xanthomonas phaseoli* in a way which might explain the subsequent decrease in pathogenicity of the bacterial pathogen in the serial passages.

What variation the progeny of single cells may undergo is another problem and one which it is hoped will be undertaken by other investigators. No generalization regarding the variation of *Xanthomonas phaseoli* could have been made, however, from single-cell studies of P(M + P)[34], as it is well known that strains of bacteria vary greatly in their ability to dissociate and there is great need for the study of many strains before it will be possible to determine the norm for a given organism in regard to variation. Furthermore the progeny of different single cells may differ one from another. Hence the study of the progeny of many single cells from each of the many strains would be necessary to complete the picture.

It is interesting to note in this connection that a number of investigators have shown that single cells may produce unlike progeny. For example, Bengsten (1), working with organisms connected with botulism, demonstrated that the progeny of a toxic single-cell culture of type C (the Saunders' strain isolated from fly larvae) contained both toxic and nontoxic individuals. The progeny of nontoxic single cells, on the other hand, never gave rise to toxin-producing cultures. Quirk (10) found that by pH control she could produce pure plate-cultures of virulent S or avirulent R forms of *Bacillus phytophthorus* from single-cell isolates of either type. McNew (8), working with the progeny of a single cell of *Bacterium stewarti* E. F. Sm. isolated by the Riker *et al.* hanging-drop method, found that weakly virulent strains developed from highly virulent cells in nutrient-dextrose broth at 25–27° C. and gradually replaced the parent type. He states that cultures containing weakly virulent cells were not appreciably attenuated unless the proportion of highly virulent parental strains fell below 21 per cent.

Sectors indicating a sudden change from the parent culture, have been observed in single-cell cultures by various workers. For example, Nirula (9), working with an unidentified organism, found in several single-cell cultures wedge-shaped sectors from which he was able to separate strains markedly different from the parent cultures. These variations remained constant and reversion could not be induced by special methods of culture. More recently Elliott and Robert (3) found sectoring in nearly all of the colonies in potato-dextrose-agar plates poured from one of the progeny of a weakly virulent single-cell culture of *Bacterium stewarti.* In this case the variation appeared to be for color only.

The filtration of P(M + P)[34] and "normal" *Xanthomonas phaseoli* covered a longer period than is customary in filtration studies. This step (Fig. 1, Series D) was not reached till late afternoon and as filtration was carried on by gravity rather than by suction, in order to avoid the possibility of pulling the bacteria through the filter, insufficient filtrate had been obtained by nightfall to make the necessary number of transfers. Hence, the filtration setup was put into a refrigerator held at 10° to 12° C. and the transfers were made early the next morning. At this temperature *Xanthomonas phaseoli* could not have grown through the filter overnight.

White variant. The white variant of *Xanthomonas phaseoli*, varying from R to S, is the one that, in the writer's experience, has been the most commonly encountered both *in vitro* and *in vivo*. The same culture may be R or S or RS at different stages of its development. It has shown marked pleomorphism in certain phases of the variation process, as is commonly reported for R forms in general. It has been most markedly brought to the fore, particularly in P(M + P)[34], by Quirk's dissociation technique with her media I to IV, but has now and then been observed as a secondary growth in old steamed-potato cultures of a number of strains of *X. phaseoli* in addition to those discussed in this paper. Colonies of the R–S white variant have appeared from time to time in years past in pH 7.0 beef-infusion-agar plates, from plants naturally infected with *X. phaseoli*. These colonies occurred much less frequently and in smaller numbers in plates poured from such plants, however, than in those from plants inoculated in the writer's association studies of *X. phaseoli* and bean virus 1 previously described (5). In the latter studies, colonies spectacularly dissociating into white and yellow forms also were isolated at times from serial passages of bean juice containing the two infective agents (5, Fig. 2).

The ability of the R–S white variant to revert in part[11] to the "typical" S yellow *Xanthomonas phaseoli in vivo*, as noted in pathogenicity tests of the filtrates of the dissociated "normal" strain, was corroborated in later unpublished studies of another strain, "Wyoming 1940," both with cultures dissociated by Quirk's technique and with potato cultures from a series in which the R–S white variant had arisen spontaneously and superseded the S yellow one. The recurrence in the writer's (M + P) serial passages (5) of bacterial symptoms on the inoculated primary leaves after an absence for 10 such passages is believed to be due to such reversion of the R–S white variant.

Reversion also takes place *in vitro*, though less readily in the writer's experience. An occasional S opaque-white colony of the R–S white variant produced "typical" S yellow growth on steamed potato and excellent S white growth on pH 7.0 beef-infusion-agar in transfers made on the same date. In such cases the medium appears to exercise a selective action on the types occurring in the colony.

Though the R–S white variant is usually but mildly pathogenic, the writer on one occasion encountered in this form of the "Wyoming 1940" strain a phase in the early stage of its transition from the S yellow type-form when it was extremely virulent and did not revert to S yellow in the host. At the opposite end of the pathogenicity scale of this white variant of *Xanthomonas phaseoli*, Wyoming-1940 strain, was a phase which has been completely avirulent in the writer's studies. It forms ± R colonies of the medusa-head type consisting of filaments (unpublished work). The writer (5) has isolated similar colonies from the serial passages of (M + P).

[11] There were always, in addition to the "typical" S yellow colonies, some of the R–S white variant present in the isolation plates from plants wherein reversion had occurred.

Unlike the starch-loving[12] S yellow type-form, the R–S white variant is very erratic in its reaction to steamed potato cylinders as a medium and frequently fails to grow at all thereon.

The studies described in this paper show that variants, both weakly pathogenic R–S white and nonpathogenic S pink, were very much more readily dissociated from the parent colony in the case of strain P(M + P)[34] than in that of the "normal" strain of *Xanthomonas phaseoli* used for comparison. In the dissociated cultures of P(M + P)[34] the mildly pathogenic R–S white variant was dominant. In the dissociated cultures of the "normal" strain on the other hand, the dominance of the "typical" virulent S yellow form was very marked. Was this dominance of the weakly pathogenic R–S white form in P(M + P)[34] due to the direct action of the virus upon the bacterium during their association *in vivo* or to its indirect influence through the stunting and physiological changes it caused in the host, conditions conducive to the production of variation in bacterial plant pathogens; to the mere presence of the virus and its competition for nutrient substances; in part to continuous plant passage *per se*[13]; or to a combination of factors?

Until we know more about the mechanism which gives rise to the results observed in bacterial or bacteria-and-virus associations, and understand more clearly the fundamental biological nature of the phenomenon of variation in the bacterial cell *per se*, we can do little more than speculate as to the answer. Too many unknown factors are involved.

Studies by Hedges and Fisher (6) relative to the effect of nutrient solutions containing varying amounts of nitrogen on the pathogenicity of the bacterium and the virus *in vivo*, either alone or associated in serial passages, showed that under the conditions of those experiments, the amount of N optimum for plant growth was optimum for both infective agents.

The demonstration of mildly pathogenic and avirulent variants of *Xanthomonas phaseoli* calls attention to the fact that the pathologist or plant breeder has solved but a part of his problem if he takes into consideration only the so-called "normal" type of this pathogen. Furthermore, the influence of certain culture media in bringing one or another form of the parasite to the fore emphasizes the importance of the medium as a factor which often affects stability of a given type of organism under cultural conditions.

SUMMARY AND CONCLUSIONS

These dissociation studies of *Xanthomonas phaseoli* were undertaken for the purpose of discovering whether changes had taken place in the bacterium during its 2 years' association with the virus *in vivo* (4, 5) which

[12] Evidenced by its luxuriant growth on steamed potato cylinders and its strong diastasic action.

[13] Parallel tests of 3 strains of *X. phaseoli* in serial passages from bean plant to bean plant of juices containing the respective isolates, have produced no diminution in severity of symptoms of bacterial infection to date, after 16 serial passages covering 6¼ months (unpublished work).

might account for its decrease in virulence and failure to maintain continuously its "typical" form in the host tissues. The writer hoped furthermore to determine whether there existed a filterable form of *Xanthomonas phaseoli* which was capable of producing mosaic.

Quirk's technique of dissociation was employed involving the use of her special media I to IV (11) and including the media commonly used for phytopathogenic bacteria. Check transfers to all media were made from young cultures.

Dissociated cultures manifested striking differences between a "normal" strain of *Xanthomonas phaseoli* and strain $P(M+P)^{34}$, a strain associated continuously for 2 years *in vivo* with the virus of the common bean mosaic. $P(M+P)^{34}$ had been isolated from the 34th serial passage of infected juice from bean plant to bean plant, *i.e.*, shortly before (a) the disappearance of all bacterial symptoms from the inoculated plants, which occurred in the 36th serial passage, and (b) the sudden onset of ultra-severe mosaic in the 38th serial passage (5).

The variations proved to be for color as well as for pathogenicity and R and S. In the dissociated cultures of $P(M+P)^{34}$, the mildly infectious R–S opaque-white and nonpathogenic S pink variants developed at the expense of the "typical" virulent S yellow form. This was best demonstrated in Quirk's media II and III. In the dissociated "normal" strain, on the other hand, with the exception of a trace on medium IV in the nonleached set the weakly pathogenic R–S white variant appeared only after leaching and was quickly overrun by the S yellow form. The S pink variant appeared in the "normal" strain only after filtering. The dissociation phenomenon and the differences between the two strains were far better demonstrated on Quirk's media II, III, and IV than on the media most commonly used for phytopathogenic bacteria.

The initial transfers of the filtrates of the leached, aged, medium-I plate cultures of each strain (Fig. 1, Series D) produced growth on Quirk's media II, III, and IV. Transfers made at the same time to 9 media commonly used for bacterial plant pathogens resulted in growth in two only, both beef-infusion broths, *viz.*, pH 6.0 inoculated with "normal" *Xanthomonas phaseoli* and pH 7.0 with $P(M+P)^{34}$. In all subcultures from the two strains in these respective broths the R–S white variant was dominant.

A barely visible, colorless, finely roughened, film-like growth was produced on medium II with the filtrate of each strain, and on medium III with that of the "normal" strain. Transfers from such films grew on a few media. Each strain produced on medium IV an initial S growth of the R–S white variant followed by the S yellow form in the case of the "normal" strain or by the S greenish yellow form with $P(M+P)^{34}$. $P(M+P)^{34}$ produced a similar growth on medium III. An S pink variant appeared in second or third lineal transfers from the bacterial suspension in the filter tube in each strain. It occurred in the form of superimposed colonies, streaks, or sectors, and it was much more conspicuous in the $P(M+P)^{34}$ strain.

The "typical" virulent S yellow form was recovered from the filtrate of each strain when transfers to Thaxter's potato-dextrose agar were made from the filter tube after 10 days' incubation. In the case of $P(M+P)^{34}$ this was accompanied by patches of S growth of the R–S white variant.

Inoculations of Stringless Green Refugee bean seedlings with the dissociated cultures of each strain showed that none of those tested, including fi'trates, was wholly avirulent. The severity of infection was directly proportional to the amount of the S yellow form in the inoculum. When the R–S white variant was dominant infection was poor or only fair, rarely visible on the inoculated leaves, and more often than not revealed only by microscopic examination of the stem. The R–S white variant from the "normal" strain filtrate reverted to "typical" S yellow *Xanthomonas phaseoli* in the host within 10 days after its use as inoculum.

Some mosaic appeared in inoculations with aged, medium-IV flask cultures of the filtrates of each strain. However, although the large number of inoculated checks showed no sign of seed infection, more work would need to be done and with a greater number of plants to prove conclusively that a filtrate of *Xanthomonas phaseoli* culture was capable of producing mosaic.

The writer concludes that the dissociation studies explain the decrease in virulence of *Xanthomonas phaseoli* during its 2 years' *in vivo* association with the common bean-mosaic virus by demonstrating the increased development of the weakly pathogenic R–S white and nonpathogenic S pink variants, which resulted in the partial subordination of the virulent S yellow typeform of the bacterium.

The writer believes that the properties of virulence, mild virulence, and avirulence are inherent in all so-called "typical" cultures of *Xanthomonas phaseoli* and that the dominance of one or another type may be determined by a number of factors both *in vitro* and *in vivo*. In this connection it seems quite probable that an associated infective agent, if present, may play, either directly or indirectly, an important rôle.

In studies such as those reported in this paper there are, in the opinion of the writer, a number of problems that require for their solution the collaboration of pathologist, bacteriologist, virologist, biochemist, geneticist, and physicist.

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ASSOCIATION OF XANTHOMONAS PHASEOLI AND THE COMMON BEAN-MOSAIC VIRUS, MARMOR PHASEOLI. III. THE EFFECT OF VARYING AMOUNTS OF NITROGEN ON PATHOGENICITY

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INTRODUCTION

Earlier studies by the senior author (2, 3) demonstrated that the virus symptoms predominated in serial passages from bean plant to bean plant of bean juice containing *Xanthomonas phaseoli* (E. F. Sm.) Dowson and the virus of the common bean mosaic, *Marmor phaseoli* Holmes. The mosaic symptoms suddenly assumed an ultra-severe form in the 38th serial passage, after two years' uninterrupted association of the virus with the bacterium in the host. Bacterial symptoms on the other hand varied from none or very mild signs of infection to good typical *X. phaseoli* lesions. No severe secondary bacterial infection occurred at any time.

Very virulent isolates of *Xanthomonas phaseoli* were obtained from time to time by means of poured plates from the serial passages as well as colonies spectacularly dissociating into the "typical" S yellow form and the mildly pathogenic white variant. (3, figure 2.) At times, notably in plates from plants with an ultra-severe form of the mosaic, no "typical" *X. phaseoli* appeared. There were, however, aberrant forms which the senior author's later dissociation studies (4) of a virulent isolate from the serial passages led her to conclude were variants of *X. phaseoli*.

That virus infections cause considerable disturbance of the physiological processes is well known (17). The common bean mosaic virus is no exception to this general rule. It is not strange, in view of this fact, that *Xanthomonas phaseoli* did not find good growing conditions in such host plants as those described in the serial passages (3).

Various investigators have reported on the effect of nitrogen on both virus and bacterial infections, *e.g.*, Spencer (9, 10, 11, 12, 13), working with tobacco-mosaic virus, found a direct correlation between virus activity and the amount of nitrogen furnished the plants and none between the growth of the host as influenced by the nitrogen supply and the increase in virus content. In other words, an excess of nitrogen beyond that producing maximum growth of the host caused maximum susceptibility.

Woods and Du Buy (16) concluded that sufficiently prolonged nitrogen starvation could prevent or retard multiplication of the tobacco-mosaic virus by reducing the activity of cyanide-sensitive respiration on which said multi-

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plication depends. The work was done with tobacco seedlings grown in sand cultures.

Considerable work has been done by McNew (6, 7) and McNew and Spencer (8, 14) on the effect of nitrogen on *Bacterium stewarti* E. F. Sm. It was found that the maximum severity of infection with this pathogen occurred with excess nitrogen and that there was a tendency for virulent strains to be replaced by attenuated ones in seedlings rendered insusceptible by nitrogen deficiency. The ability of a strain to use inorganic nitrogen was essential to its virulence, weakly virulent strains being obligate users of organic nitrogen (7), and there was a stronger tendency for the development of virulent strains in nitrogen-fed plants than in those deficient in nitrogen.

Clayton (1) found that high nitrogen fertilization combined with low topping of tobacco plants, a cultural practice in some regions, favored maximum development of wildfire by increasing susceptibility to water-soaking which he found to be a necessary condition for the occurrence of the destructive epidemic type of wildfire. Experimental field plants with low nitrogen fertilization were difficult to water-soak and were resistant to *Pseudomonas tabaci* (Wolf and Foster) Stapp in both sandy and more fertile soils.

A comprehensive review of the work of numerous investigators on the effect of nutrition on the susceptibility of plants to disease has been published by Wingard (15).

The senior author's earlier work (3) had been carried on with pot experiments using sterilized composted soil. The present investigations were undertaken with the purpose of studying the effect in sand cultures of varying amounts of nitrogen on the disease complex and on the activity of the two infective agents *per se*. A preliminary report has been published (5).

MATERIALS AND METHODS

Bean seedlings were grown in sterilized, washed quartz sand and thinned to 3 plants per 4-inch pot in experiment I, to 4 plants in experiment IV. Later 6-inch pots with 4 plants each were used. The pots were supplied with saucers.

The inocula consisted of (a) the crushed tops of infected plants from the serial passages of bean juice containing the bacterium and the virus, (b) pure single colony cultures of *Xanthomonas phaseoli* growing on steamed potato cylinders, and (c) crushed infected trifoliate leaves or plant tops from serial passages of bean juice containing the mosaic virus.

The inoculum was rubbed on the under surface of primary leaves when they were $\frac{1}{2}$ to $\frac{2}{3}$ full grown, at which time the seedlings were usually about 10 days old and no trifoliate leaves had unfolded. No carborundum was used.

Feeding of the sand cultures began one or two days after inoculation and consisted of the addition of 100 ml. nutrient solution to each pot 6 times weekly. The plants were watered with tap water as needed.

The nutrient solutions containing low, medium, and high nitrogen were

made up according to the formulae published by Spencer (11, p. 770) in his paper on the effect of host nutrition on concentrations of tobacco-mosaic virus. The solutions contained 9.8 p. p. m., 200 p. p. m., and 2000 p. p. m. nitrogen, respectively.

For comparison, inoculations were made on plants growing in sterilized composted soil.

Noninoculated plants as checks on seed infection were grown both in sterilized sand watered with the nutrient solutions and in sterilized composted soil. They were kept isolated from the inoculated plants.

The line of Stringless Green Refugee bean used in these investigations is the same as that employed in all previous association studies by the senior author (3, 4). The seed was collected by W. J. Zaumeyer from healthy plants in his breeding plots in Greeley, Colorado, or from the writer's healthy noninoculated checks from the same seed source. The same is true of U. S. No. 5 Refugee.

Abbreviations

M = bean mosaic virus.

P = *Xanthomonas phaseoli.*

P. var. = a yellow variant of *X. phaseoli,* differing from the "normal" strain in colony characters (3, p. 665, footnote 2), growth on steamed potato, and pathogenicity when first isolated. It usually reverts to the "normal" form in later passages through the plant. The strain of P. var. used in these fertilizer experiments was isolated 4 years previously from the petiole of a trifoliate leaf of Stringless Green Refugee inoculated by rubbing the primary leaves with *X. phaseoli* Cuba 1934 strain + mosaic virus, and it was one of the strains used in the serial passages (3, p. 679, footnote 12). "Typical" *X. phaseoli* was masked in the blade of the same trifoliate leaf and isolated therefrom.

$(M+P)^{57}$ and $(M+P)^{64}$ = juice from the 57th and 64th serial passages of bean juice containing the mosaic virus + "typical" *X. phaseoli.*

$(M+P.\ var.)^{66}$ = juice from the 66th serial passage of bean juice containing the mosaic virus + the yellow variant of *X. phaseoli* cited above.

$P(M+P)^{34}$ = an isolate of *X. phaseoli* from the 34th serial passage of the two infective agents. Used for dissociation studies (4).

EXPERIMENT I

Inoculations with $(M+P)^{57}$

The inoculum consisted of crushed tops of plants from the 57th serial passage of bean juice containing both infective agents. One hundred per cent of these plants had severe mosaic but no signs of bacterial infection at the time their tops were used as inoculum, *viz.,* about 3 weeks after inoculation. Mild bacterial stem infection was demonstrated later by microscopic examination.

This experiment was comprised of 8 sets of five 4-inch pots containing

3 Stringless Green Refugee seedlings each, 6 sets with sterilized washed quartz sand, and 2 with sterilized composted soil. Before the unfolding of the first trifoliate leaf, 4 sets (60 plants) were inoculated on the primary leaves. The remaining 4 sets were held as checks. Two days later (March 5) the feeding of the sand cultures with 3 nutrient solutions was begun. These were supplied at the rate of 100 ml. nutrient solution per pot 6 times a week and the 6 sets so fed, including inoculated plants and checks, were designated as "low," "medium," and "high N," respectively.

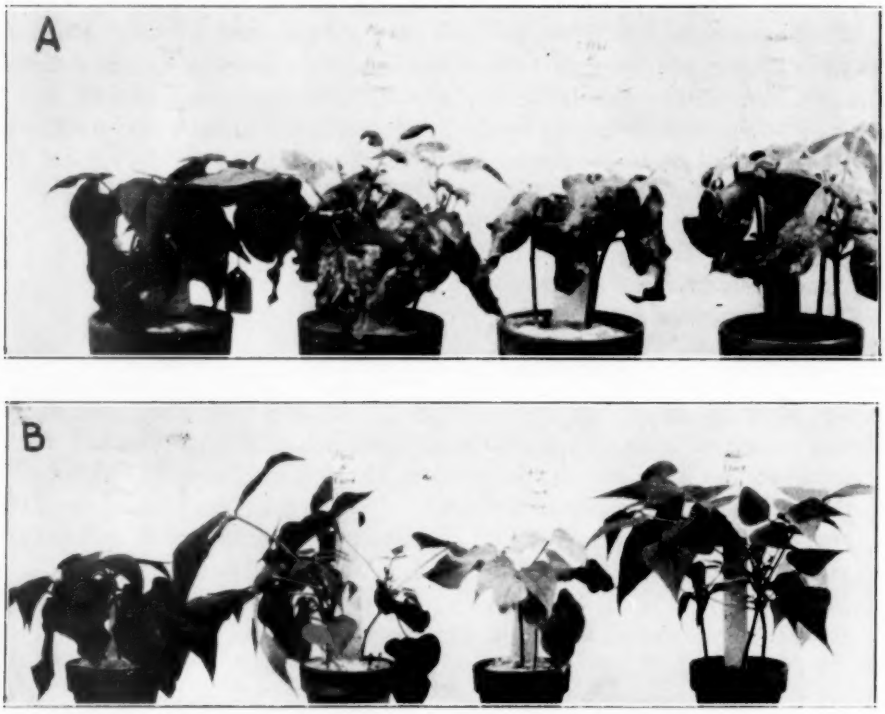

FIG. 1. A. Stringless Green Refugee bean seedlings inoculated with the crushed tops of plants from the 57th serial passage of bean juice containing *Xanthomonas phaseoli* and the common bean mosaic virus, *viz.*, $(M+P)^{57}$. Experiment I. Left to right: Sand cultures watered with nutrient solutions containing high N (2000 p. p. m.), medium N (200 p. p. m.), and low N (9.8 p. p. m.), and controls in composted soil. Note striking *Xanthomonas phaseoli* infection in medium N. Photographed two weeks after beginning experiment. (Compare noninoculated checks, Figure 1, B.) B. Noninoculated checks, watered with nutrient solutions as in A.

The most interesting thing about these inoculations with $(M+P)^{57}$ was the spectacular outbreak in the medium N set of *Xanthomonas phaseoli* which had been masked in the inoculum. There were very conspicuous typical lesions on the rubbed primary leaves of 100 per cent of the plants (Fig. 1, A). In the high and low N sets, on the other hand, bacterial infection was confined to scarcely discernible water-soaked spots along the veins. In the composted soil set, bacterial infection was somewhat more distinct but by no means conspicuous. Isolates from the 3 nitrogen sets were tested for pathogenicity in experiment III.

Virus infection, manifested by curled trifoliate leaves and much dwarfing, was most striking in the medium N and composted soil sets where there was 100 per cent severe mosaic in 12 days. It was somewhat more severe in the composted soil set. There was also 100 per cent virus infection in the high and low N sets, but it was much less striking.

Expressed juice from each set was tested for pathogenicity of the two infective agents therein (See experiment II).

The effect of the different nutrient solutions on plant development was clearly visible in the noninoculated checks in 5 days after the feeding began. The medium N set was normal in color and size, closely resembling the plants in composted soil. The high N set was dark green and dwarfed, and the low N set was a decidedly yellow green, spindling, and, on this date, the tallest of the three sets of sand cultures. The medium N set outgrew it later (Fig. 1, B). The check plants remained free from infection. None of the inoculated plants ever blossomed.

EXPERIMENT II

Tests of Virulence of the Expressed Juice from Plants Inoculated with $(M+P)^{57}$ and Receiving Varying Amounts of Nitrogen

To test the infectivity of the juice of the inoculated plants in the 3 sand culture sets in experiment I after 3 weeks of feeding with varying amounts of nitrogen, rub-inoculations were made on the primary leaves of 10-day-old Stringless Green Refugee seedlings and, for comparison, on the mosaic-resistant U. S. No. 5 Refugee. A set of each variety was also inoculated with juice from the inoculated plants grown in sterilized composted soil. There were 20 plants in each unit, growing in 6-inch pots of composted soil.

The results indicated that there had been an inhibiting effect of the high and low nitrogen on the bacterium, since, with a single exception,[2] no visible bacterial infection occurred on either bean variety inoculated with juice from plants in the high or low N sets.

On the contrary 100 per cent of the primary leaves of both bean varieties became infected with *Xanthomonas phaseoli* after they were rubbed with the infected juice of plants from the medium N and composted soil sets. There was also much severe secondary infection on the stems of the mosaic-resistant U. S. No. 5 Refugee. Fifteen out of 20 plants were girdled and broken over in two months.

The virus, on the other hand, was not permanently affected by any of the nitrogen concentrations. In 14 days 100 per cent of the Stringless Green Refugee in all sets were mosaiced. This was likewise the case with plants inoculated with juice from the composted soil set. No mosaic appeared on the U. S. No. 5 Refugee.

Neither bacterial nor virus infection appeared on the noninoculated checks of either variety.

[2] One plant of U. S. No. 5 Refugee rubbed with inoculum from the high N set became infected.

EXPERIMENT III

Tests of Virulence of Xanthomonas phaseoli Isolates from Plants Inoculated with $(M+P)^{57}$ and Receiving Varying Amounts of Nitrogen

Isolates of *Xanthomonas phaseoli*, "typical" in appearance in poured plates and on steamed potato cylinders,[3] were obtained from the high, medium, and low nitrogen sets in experiment I about 3 weeks after inoculation. Dissociating colonies with many white sectors were also present in poured plates from the low N set.

Comparative inoculations were made on 60 rapidly growing 12-day-old Stringless Green Refugee seedlings in sterilized composted soil using steamed potato cultures from single "typical" colonies as inoculum.

The isolate from the medium N set was by far the most virulent and infection progressed so rapidly that 19 out of 21 plants inoculated were girdled and broken over in 4 weeks, and none of the plants fruited. This was much more severe than the bacterial infection produced by the expressed juice from the medium N set.

Unlike the bacteria in the juice-inoculations described in experiment II, the pure cultures of *Xanthomonas phaseoli* from the high and low N sets were also very virulent. In all 3 sets there was 100 per cent excellent infection with a high percentage of girdled, broken-over stems. The greater pathogenicity of these isolates as contrasted with that of the bacteria in expressed juices from the same source is notable and probably due to a number of factors.

The checks remained healthy and produced a fine crop.

EXPERIMENT IV

Inoculations with $(M+P)^{64}$, Xanthomonas phaseoli, and Mosaic Virus

This experiment, begun June 25, differed from experiment I in (a) the use as inoculum of plants from a serial passage showing 100 per cent visible infection with both pathogens—bacterial as well as virus, (b) addition of a second and third set of inoculations with *Xanthomonas phaseoli* and the mosaic virus, respectively, (c) the use of a far larger number of plants.

The inocula used were as follows:

1. $(M+P)^{64}$: Crushed tops of plants from the 64th serial passage of bean juice containing the two associated infective agents; 100 per cent visible infection with both pathogens. The diseased plant material was collected 18 days after the bean seedlings were inoculated.

2. A sterile distilled water suspension of 3-day-old cultures on steamed potato cylinders of *Xanthomonas phaseoli* Z strain, long in stock, virulent, and with no history of association with the mosaic virus.

3. Crushed typical mosaic-infected trifoliate leaves from plants inocu-

[3] Copious S yellow ± syrupy growth, so filling the water at the base of the cylinder that the tubes could be turned upside down with impunity.

lated 18 days previously with the juice of infected trifoliate leaves selected from diseased plants in serial passages of mosaic virus-containing juice.

In the sand-grown sets, each unit (*Xanthomonas phaseoli*, low nitrogen, *e.g.*) contained 100 plants. In the composted soil series, because of a shortage of seed, this number was reduced to 76 in the inoculated set and to 64 in the check set. A total of 1492 Stringless Green Refugee bean seedlings were involved.

The groups treated with different inocula were kept some distance apart. The noninoculated checks on seed infection were isolated.

Four plants were grown per 4-inch pot. The nutrient solutions and schedule of applications used were the same as in experiment I.

$(M+P)^{64}$ Inoculations

Bacterial infection: In the series inoculated with $(M+P)^{64}$, the most severe initial bacterial infection was in the composted soil; the medium N series was a close second. Infection by *Xanthomonas phaseoli* was excellent in the high N set but developed much more slowly. There was ultimately 100 per cent bacterial infection of the primary leaves in all four sets. In those plants suffering from nitrogen starvation (9.8 p. p. m. N), the initial lesions increased but little in size and soon dried up. However, the bacteria reached the stems of at least 65 per cent of the plants, as determined by hand lens examination.

Virus infection: There was severe development of mosaic, with much dwarfing and many curled trifoliate leaves, in both the medium N set and the composted soil, progressing a little more rapidly in the former but reaching 100 per cent in both in 14 days. There was also 100 per cent mosaic in the low N set but it had developed much more slowly. In the high N set very few plants showed signs of mosaic infection other than dwarfing.

The $(M+P)^{64}$ group in experiment IV showed, in 11 days, much more dwarfing (all sets) than did the plants inoculated with mosaic virus alone.

The plants at times appeared to be overwatered by the 100-ml. nutrient solution applied 6 times a week. This, as might be expected, was most marked in the high N set where the plants, in addition to being stunted by the inoculation, were dwarfed by the excess nitrogen.

Xanthomonas phaseoli Z strain inoculations

By far the most severe bacterial infection was obtained in the medium N set. The difference between this and all other sets in the *X. phaseoli* group was conspicuous. There was 100 per cent heavy infection of the inoculated primary leaves and 68 per cent stem infection in the medium N set.

In the low N set, good but scattered lesions were on the primary leaves, and 62 per cent visible secondary infection on the stems. Yellow bacterial ooze was produced in 32 per cent of the infections by the 29th day.

In the high N set, a high percentage of the rubbed primary leaves had a very slowly developing infection, although at the end of two weeks these

plants had the fewest bacterial lesions of any set in the *X. phaseoli* group. There was never any visible secondary infection on the stems which were under observation for 38 days.

In composted soil there was only scattered infection on the primary leaves but 42 per cent visible stem infection in 30 days.

Mosaic virus inoculations

Virus infection in this group was very poor as a whole. The best, a little mild mosaic with some curling of trifoliate leaves, appeared in the sand with medium N and the composted soil sets. There was some dwarfing in the low N set, which may have been due to the virus, but no other mosaic symptoms appeared. There was little if any virus infection in the high N set. These results were puzzling and might have been laid to the failure to use carborundum had it not been for the fact that in the serial passages of juice containing the virus alone, infection had never fallen below 80 per cent and in 7 of the 10 passages completed at this time[4] had reached 100 per cent good but not severe mosaic infection without the use of carborundum.

The most striking thing about this virus group was the outbreak of typical *Xanthomonas phaseoli* infection in 73 per cent of the plants in the medium N set though no bacterial infection had been observed in the plants in the mosaic-virus serial passages from which the inoculum was obtained. A little bacterial infection also occurred in the high N set but none in the low N or soil sets. Apparently *X. phaseoli* was masked in the inoculum and, as in experiment I, it had been stimulated by the 200 p. p. m. N in the medium N set.

Checks

There was no sign of virus or bacterial infection in any of the 300 noninoculated check plants growing in sand or in the 64 in composted soil. The optimum growth was in the medium N set.

Overwatering

It was apparent that 100 ml. of nutrient solution 6 times a week constituted overwatering for the dwarfed plants, particularly those in the high N sets of all groups including the noninoculated checks. On the other hand it was sometimes not quite enough for the groups in which the plants made a normal amount of growth, notably the *Xanthomonas phaseoli*, virus, and check groups in the medium N set.

Beginning with the 21st day the high N sets in all groups were fed every other day and water was given here and there as needed.

EXPERIMENT V

Inoculations with (M + P. var.)[66], Xanthomonas phaseoli and Mosaic Virus

As previous experience had shown that the dwarfed sets were frequently

[4] The 10th serial passage had been made the day following the inoculations in this virus group.

overwatered, experiment IV was repeated the first of October, using 6-inch pots[5] with 4 plants each and adding water here and there as needed. As a result none of the sets showed ill effects of overwatering. Sixty plants comprised each of the sand units and 48 plants the composted soil units. The feeding began October 5, the day after inoculation.

Aside from the fact that the virus inoculum used in this experiment was more virulent, the results previously obtained were confirmed using another set of inocula as follows:

1. (M + P. var.)[66]: Tops of plants from the 66th serial passage of juice containing the mosaic virus and the yellow variant of *X. phaseoli* described in an earlier paper (3). One hundred per cent of the plants of the 66th serial passage had excellent mosaic and scattering mild *X. phaseoli* infection. The material was collected three weeks after inoculation.

2. *Xanthomonas phaseoli:* A sterile distilled water suspension of cultures on steamed potato cylinders of the Wyoming 1940 strain, a very virulent isolate recently obtained from a bean pod and having no history of association with the mosaic virus.

3. *Virus inoculum:* From serial passages of juice containing mosaic virus. They had been started 8 months earlier by the use of seed-infected plants as inoculum. The plants had been inoculated 4 weeks previously.

Crop

Unlike the previous experiments, experiment V was continued long enough to form an estimate of the crop, that is, until the plants were 7½ weeks old. In all sets the crop was much reduced by infection, most in the (M + P. var.)[66] group which did not even blossom, next in the virus group, least by *X. phaseoli.*

Only the checks were in condition to mature all the pods on the plants at the time the count was made. They produced about 1.4 times as many in composted soil as in the medium N set, and very few in low and high N, least in the latter.

DISCUSSION

Although various investigators have reported on the effect of nitrogen on the activity of plant pathogens, none, to the authors' knowledge, have worked on its effect on associated infective agents. The present investigations were carried on from that point of view.

In the studies here reported, the results may have been somewhat different had not root nodules formed notwithstanding the sterilization of the washed quartz sand in which the seedlings fed with nutrient solutions were grown. It is quite conceivable that 200 p. p. m. N would not have been sufficient for 2 pathogens in addition to the host without the extra N supplied by the root nodules. Scattered small nodules and a few large ones were formed in plants in this medium N set. In the high N (2000 p. p. m.) set, there were scattered small nodules and in the low N (9.8 p. p. m.) set, scat-

[5] Six-inch pots contain 3½ times as much soil as 4-inch pots.

tered large ones. L. T. Leonard states (oral communication) that under ordinary greenhouse conditions it is almost impossible to exclude the nodule bacteria when growing many species of legumes.

A comparison of the bacterial infection produced by inoculations with (A) the expressed juice from plants of the 3 nitrogen sets of $(M+P)^{57}$ inoculations in experiment I (See experiment II), and (B) steamed potato cultures of isolates from the same nitrogen sets (See experiment III) is illuminating. The lesser pathogenicity of the bacterium in the expressed juice inocula is believed to be due (a) to its long *in vivo* existence in the much stunted plants of the serial passages in association with the virus, in which circumstances a tendency to dissociation into less virulent and avirulent variants has been demonstrated (4) and (b), in the case of the bacterium in the juice from the high and low N sets, to the additional inhibiting influence of excess and deficient nitrogen. On the other hand the greater pathogenicity of the pure cultures of the isolates is in all probability due to the preliminary building up of their virulence on a favorable culture medium (steamed potato cylinders) which favors the virulent S yellow form and tends to repress the mildly pathogenic and avirulent variants.

Unfortunately none of the available virus inoculum was absolutely free from the suspicion of the presence of masked *Xanthomonas phaseoli*. Just before making the last set of inoculations in the virus group (experiment V), bacterial infection broke out for the first time in the source of inoculum, *viz.*, the mosaic virus serial passages carried on for 8 months. It was such an outbreak of *X. phaseoli* in Zaumeyer's tests of hybrids for mosaic resistance that had led to the first association studies by the senior author (3).

There was no mosaic-infected seed at hand from which a fresh start towards obtaining virus inoculum could be made. Hence the inoculum for the virus group cannot be considered assuredly pure virus comparable to pure cultures of *Xanthomonas phaseoli*. As for the virus infection in the virus group, it is possible that better results would have been obtained with the use of carborundum although 100 per cent good but not severe mosaic infection had occurred without its use in 10 out of 14 serial passages of the virus completed up to this time. Furthermore, in none of the 14 passages had infection fallen below 80 per cent.

SUMMARY

The effect of varying amounts of nitrogen, 9.8 p. p. m., 200 p. p. m., and 2000 p. p. m., was tested on Stringless Green Refugee bean seedlings grown in sand and inoculated with (a) juice from serial passages of bean juice containing *Xanthomonas phaseoli* and the common bean mosaic virus, (b) pure, single colony cultures of *X. phaseoli*, and (c) juice from serial passages of bean juice containing the common bean mosaic virus. Comparative inoculations were made on plants grown in sterilized composted soil and noninoculated plants, grown in each substratum, were held as checks on seed infection.

Of the three concentrations of nitrogen tested, medium N (200 p. p. m.)

produced the most intense development of both virus and bacterium in every case. This was also optimum for growth of the host.

Pathogenicity tests of juice from all sets of plants inoculated with $(M+P)^{57}$ in experiment I showed that the virus had not been permanently inhibited by any of the three N-concentrations, inasmuch as the juice from all sets produced 100 per cent good mosaic when tested on plants grown in composted soil. On the other hand there had been marked inhibition of the bacterium by both low N (9.8 p. p. m.) and high N (2000 p. p. m.).

When, however, isolates of the bacterium from the same three nitrogen sets were first grown on a favorable culture medium (steamed potato cylinders) and then used as inoculum, all were very virulent although the isolate from the medium N (200 p. p. m.) was notably more so.

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TYPES OF DEFENSIVE REACTIONS IN PLANTS

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By a defensive reaction we understand a vital process initiated in a host by a pathogenic agent and directed more or less specifically against that agent itself. In a book recently issued, the writer (6) has endeavored to discuss in detail the various types of defensive reactions in plants. It is the purpose of this article to represent certain points of view as set forth in that treatise.

The defensive reactions in plants may be broadly classified as follows: 1. According to the object against which they are primarily directed; 2. According to the life history of the infected plant; 3. According to the intensity of the reaction.

The object of the defensive reactions. According to their object the defensive reactions may in turn be grouped in three subdivisions, *viz:*

a. The anti-infectional reactions, which are directed against the parasite itself and endeavor to weaken or annihilate it;

b. The antitoxic reactions, which are not primarily directed against the parasite, but against its metabolic products, especially its toxins, and, furthermore, against the necrotic products of the affected cells and tissues, which they tend to render harmless;

c. The induced tolerance, which (contrary to the previously given definition) is directed neither against the parasite nor against its metabolic products; it acts rather against the sensitiveness of the host plant itself to these pathogenic agents. The host plant undergoes a desensibilization by virtue of which it no longer reacts to the pathogenic agent. The plant tolerates the latter without manifest symptoms and, although infected, does not become diseased.

The life history of the infected plant. The capacity for reacting defensively is either spontaneous (autonomous) or acquired (induced). The first is hereditary, endowed by nature; the second remains merely potential, latent, until it is activated by a primary infection; by withstanding this the plant's readiness to resist is sensitized so that only then does it develop its full defensive capacity.

The intensity of the defensive reactions. Reactions are either normergic or hyperergic. In normergic reactions (which need not necessarily be merely defensive) cause and effect bear a certain proportionate ("normal") relation to each other. When parasite and host bear such a normal relationship, it is termed eusymbiontic; although each partner is in conflict with the other, they still continue together in a kind of parasitic community, which leads to disease. The hyperergic reactions represent the other extreme. The ratio between cause and effect becomes disproportionate. A host-parasite relationship of this kind is termed parabiontic; the two part-

ners do not tolerate each other; they react transversely to each other, with the result that within a few hours necrotic alterations in the infected cells, or in the adjoining noninfected cells, manifest themselves and rapidly cause death.

In accordance with these viewpoints it was attempted to classify in table 1 the different types of defensive reactions so far as they have been exactly determined in plants.

I. The plasmatic anti-infectional reactions. These represent the normergic type of anti-infectional defense, most easily traceable in cases of chronic infections, when parasite and host are in contact over protracted periods. Similar reactions doubtless occur in the course of acute disease, but, being more rapid and irregular, are more difficult to observe and to interpret. They operate along three lines.

TABLE 1.—*The types of defensive reactions in plants. After Gäumann (6)*

Object of the reaction	Historical development	Intensity	Type of reaction
The pathogen itself (anti-infectional reactions)	Autonomous	Normergic	I. Plasmatic anti-infectional reactions
		Hyperergic	II. Necrotic reactions
	Induced	Normergic	III. Premunity
The metabolic products of the pathogen (anti-toxic reactions)	Autonomous	Normergic	IV. Histogenic demarcations
		Hyperergic	V. Gummous demarcations
The innate sensitiveness of the host (induced tolerance)	Induced	Normergic	VI. Desensibilization

a. They alter and weaken the invading parasite. For example, ***Bacterium radicicola*** Beij. destroys the root cells of weakened Leguminosae. In vital plants, however, the bacterium is altered under the influence of the defensive reactions: the host cells remain alive and retain ability to divide despite being flooded by the bacteria (17). To take a mycological example: when lateral hyphae of intercellular fungi enter the host cells, the hyphae generally stop growing and become knotty and deformed. Since they dissolve and absorb the contents of the host cells, their peculiar form is regarded as fungoid ("haustoria"). This assumption is not unreservedly right; when the same fungi are grown on dead tissues of the same hosts, they form no such "haustoria" in the host cells, although they extract their nourishment from the latter. Hence, the haustoria are not a growth-form determined by the fungus, but are reaction-products between the defensive reactions of the host cells and the counter-reaction of the intruding hyphae.

b. They localize the intruding pathogen. Only a few infectious diseases of plants are systemic; most of them are localized (*e.g.*, leaf-diseases).

We may assume that the reason lies in the functional defense of the host cells, which causes an exhaustion of the invading agent and finishes by stopping and localizing the infection. When the same leaves are first killed by freezing, the same parasites (*e.g., Cercospora beticola, Venturia inaequalis, Diplocarpon rosae*) are able to grow right through them without restraint.

c. They digest and eliminate the intracellular parasites. Thus, haustoria are in time corroded and live for a relatively shorter period than the intercellular hyphae. The intracellular bacteria of the root-nodules (Fig. 1) and the intracellular hyphae of mycorrhizae (Fig. 2) furnish further examples of these digestive processes.

FIG. 1. Digestion of *Bacterium radicicola* Beij. in the root-nodules of *Lupinus albus* L. 1500×. After Schaede (14).

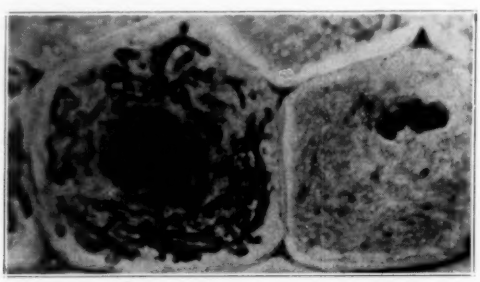

FIG. 2. Digestion of *mycorrhiza* in the roots of *Platanthera chlorantha* Cust. Left, a cell rich in content, showing incipient digestion. Right, a cell poor in content, showing an advanced stage of digestion. 160×. After Burgeff (4).

These three normergic principles (weakening, localizing, and eliminating the parasite) are based on biochemical reactions; they are borne by agglutinins, lysins, etc., as is proved by appropriately selected examples. Compared with the anti-infectional reactions of the human body their efficiency is low. Not only do they generally fail to prevent the infection, but they also are mostly unable to eliminate the invading parasite; hence, they do not lead to an autosterilization, to healing of the infected tissues; these remain, as a rule, affected for the rest of their life. Accordingly plants in general do not recover from their infectious diseases.

The cause of this inefficiency doubtless is to be attributed to the extreme simplicity of the mechanism of the plasmatic defensive reactions. In the

human and animal body, invading germs are combatted not only by the biochemical action of agglutinins, lysins, etc., but mainly by special resistance cells, *viz.*, by the mobile leuco-phagocytes and by the stationary reticuloendothelial system. Above all, the mobile defensive system enables the host to eliminate the pathogen within a short time, by assembling at the point of danger hundreds of thousands of fighting-cells, which eliminate, by fighting one cell against another, the invading germs. Such mobile fighting cells are unknown to the plant; hence it is unable to mobilize at the point of infection the full resistance-potential of entire tissues or organs. Each infected cell-group must resist by itself, independently and without help from other cells. Hence, it is not surprising that the normergic plasmatic defense system in plants meets with such a low degree of success, merely localizing, but not healing, the infections.

II. The necrotic reactions. These represent the hyperergic type of reactions and are caused by a hypersensitiveness of the host cells to the emanations of the parasite. Their consequence for the parasite-host system are diametrically opposed according to the biological type of the parasite, which may be either necrotrophic or biotrophic.

Necrotrophic parasites (*e.g., Botrytis cinerea, Valsa, Corticium vagum*) are able to feed on dead substances of the host. If, therefore, the host cells die at their approach, the further development of the parasite is facilitated.

Biotrophic parasites (*e.g., Synchytrium endobioticum,* mildews, rusts) on the contrary, can only nourish themselves from the living substance of the host. Hence if their intrusion is met by hyperergic reactions of the host cells and if the latter perish, the further development of the parasite is rendered impossible, a sort of scorched earth strategy in nature. Accordingly, necrotic reactions are not actually defensive reactions, but rather the evidence of the highest susceptibility or hypersensitiveness. But since the invading parasite is destroyed simultaneously with the death of the host cells, these hyperergic reactions have an anti-infectional effect: hypersensitive individuals are resistant to biotrophic parasites because they are oversusceptible.

The timing of the hyperergic response varies. The epidermal cells of red clover perish at the very first contact with the infectious hyphae of *Erysiphe martii* (Fig. 3, A and B), while in Kanred wheat the symptoms of the hyperergic effects appear only after some days (Fig. 3, C).

III. Premunity. In the reactions so far dealt with, the full readiness and capacity for reaction were innate to the individual (autonomous reactions). Among the numerous reactions which are initiated in the invaded individuals by the pathogen, and which mostly lead to a damaging of the former, there are nevertheless some which occasionally produce the opposite result. Instead of weakening the host, the attacking pathogen stimulates or sensitizes it, activating in it a resistance power which was previously only latent or insufficient (induced defensive reactions).

In the field of induced defensive reactions, however, the premises in phytopathology and in human and veterinary medicine are rather different. In vaccination, it is the recovery from an artificially introduced infection, which leads to an alteration in the reaction-norm of the organism. The pathogen disappears from the body, which however retains an increased resistance power against a later re-infection.

This form of acquired immunity can scarcely be expected in plant life, because plants cannot, as a rule, be entirely freed of infection. They are, however, capable of attaining a different type of immunity, a type known in human medicine (dermatology), but of no great significance there; this is infectionary immunity or premunity, based on the fact that an organism or certain of its tissues, cannot be effectually superinfected by the same or

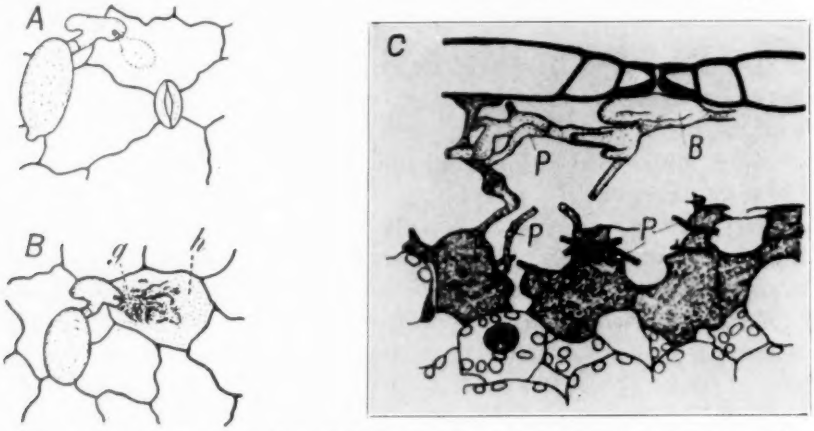

FIG. 3. A and B. Infection of *Trifolium pratense* L. by *Erysiphe martii* Lév. A. A susceptible variety of clover, which only reacts normergically, and in which the parasite can form a haustorium, without causing any apparent disturbance. B. A hypersensitive variety, in which the invaded epidermal cells undergo a sudden necrotic degeneration and perish together with the intruding parasite. *g*, infection hypha; *h*, necrotic host-cell. 385×. After O. F. Smith (15). C. Hyperergic reaction of Kanred wheat to an infection of *Puccinia graminis tritici* Erikss., biotype I. *B*, substomatal vesicle; *P*, fungus hyphae, partially abortive. Approx. 350×. After Allen (1).

by a related pathogen, so long as the original infection exists. In acquired immunity, the curing of a primary infection provides protection against a re-infection, whereas, in premunity, a still-existing primary infection provides protection against a super-infection.

Accordingly, premunity, like acquired immunity, is based upon an alteration in the host, effected by pre-infection, through which the host has gained a resistance power which it did not originally possess. In contrast to acquired immunity, the alteration in the host is maintained in the case of premunity only as long as the pre-infection lasts.

Premunity can, according to the field of its action, produce either a local immunity from infection or a local immunity from disease.

A classical example of a local immunity from infection is furnished by Bernard's experiments with orchid mycorrhiza (2). If young orchid em-

bryos be infected by a less aggressive strain of mycorrhiza, the hyphae penetrate at the suspensor region to a depth of several cell layers; their further progress is then checked. If, however, after some days, the pre-infected embryos be subjected to a virulent fungus strain, one so virulent as would normally kill the embryo, this fungus is no longer able to enter the infected region. When, however, in later stages, the embryo produces bristles, the lethal infection can enter through these. The embryo's readiness to resist infection has thus been only locally sensitized. This local premunity is group-specific; if it is initiated by a weak strain of *Rhizoctonia repens* Bern., it is also effective against aggressive strains of *Rhizoctonia mucoroides* Bern. and of *R. lanuginosa* Bern.

Premunizing experiments made by Salaman (11, 12) with the X-virus may be mentioned as an example of an induced local immunity against disease. A number of tobacco plants were preinfected with the weakest G-strain, the symptoms of which were so mild as to cause only the suspicion of a mottling. The plants were then superinfected by the virulent S-strain in consecutive groups, at 24-hour intervals. In the superinfections of the first 4 days the S-strain penetrated and the plants became diseased according to the S-type. The group superinfected on the fifth day evinced a partial resistance to the S-strain-disease, while the group treated on the eighth day no longer showed any symptoms of S-disease; the plants in this group reacted only to the G-strain: in them, the S-virus could not develop beyond the point of infection.

Thus, previously infected individuals remain susceptible to superinfection. After a period of latency (4 days), however, they become capable of arresting the general spreading of the superinfected virus; they localize it, and therefore become resistant to disease, just as in the case of human beings who have suffered from diphtheria. The induced protection is specific in such cases and is not effective against Y- and ring-spot viruses. In other cases, however, the premunity is group-specific and, furthermore, its effect can reach a depth of 7 cell layers beyond the pre-infected zone, thus leading to a local tissue-immunity (5, 7, 18).

IV. Histogenic demarcations. The defensive reactions so far dealt with are exclusively of anti-infectional type, being directed against the parasite. As, however, they generally succeed in merely weakening and localizing, but not eliminating it, a chronic disease-focus results. Consequently, the host organism has the new task of seeking to protect itself against the pathogenic metabolic products emanating from the focus; especially against the toxins of the parasite and the necrotic products of its own invaded cells. This protection is effected by means of the antitoxic reactions; their aim is not to prevent infection, but to prevent disease.

In the human system such antitoxic protection is provided biochemically by means of specific antitoxins, which neutralize the pathogenic agent (destroying it functionally), and histologically by means of demarcating tissues.

In plant pathology antitoxins have not yet been found, but numerous demarcation reactions are known; histogenic demarcations (Fig. 4) may be mentioned as a normergic example, and gummous demarcations as an hyperergic example.

To judge by such investigations as have been made on histogenic demarcations, they possess chiefly antitoxic, not anti-infectional properties; therefore the parasite is sometimes able to penetrate them. The reaction chain is not initiated by the parasite itself, but by the necrosis caused by it. To this extent, demarcation reactions are nonspecific, being indifferent to the particular source of the necrosis, which may be one of the following: necrogenic products of the lesions, toxins of the parasite, or any other organic or inorganic poisons. Similarly the mechanism of demarcation is

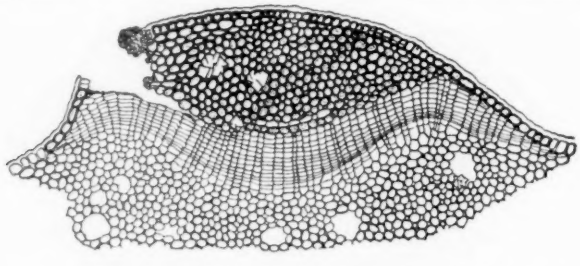

FIG. 4. Focus of *Clasterosporium carpophilum* (Lév.) Aderh. on branch of almond, *Prunus amygdalus* Stok., confined by a demarcation tissue. 60 ×. After Samuel (13).

nonspecific; it corresponds to the widespread manifestation that local lesions produce a localizing tissue in plant bodies (dehiscence tissue, scar tissue, etc.).

V. Gummous demarcations. An example of the antitoxic effect of a hyperergic gummosis may be found in the silver-leaf disease of plum trees. If a root or the trunk be locally infected by *Stereum purpureum*, toxins of this agent are carried with the sap into the upper regions of the tree, where they cause cellular pathological changes (silver-leaf effect). In severe cases the disease can lead to the death of the tree within a few years.

In certain varieties of plum trees, the capacity of resistance to disease depends upon the readiness of the woody tissues to respond with hyperergic antitoxic reactions. The Victoria plum reacts weakly and therefore falls an easy victim to the disease (3). On the other hand the trunks of Pershore plum respond with an ample exudation of gum, which forms an impassable barricade to the toxins, similar to the reaction zone in beeches (Fig. 5). The regular formation of this antitoxic zone in the beech possibly provides the reason why this species is not liable to silver-leaf disease, notwithstanding its continual contamination by *Stereum purpureum*.

Both plum varieties mentioned are liable to infection; but the Pershore variety is better able to resist disease because it is able to prevent the flooding and poisoning of its tissues by the pathogenic agents emanating from the focus.

VI. Induced tolerance. Even when a plant is unable to localize the pathogenic agent (the parasite or its toxins) and is consequently flooded by these, its condition is not necessarily hopeless. It can, in certain cases, resort to a new form of disease prevention, by simply ignoring the agent. Even though flooded by the pathogen the plant does not react to it, in short, it becomes tolerant (induced tolerance).

Induced tolerance has one feature in common with the antitoxic reactions, in that it shuts out the result of the infection; but, not as the latter does, by neutralizing or localizing the harmful agent, but rather by adopting the opposite extreme of behavior. It effects a desensibilization of its own system, which no longer answers to the pathogenic agent, and there-

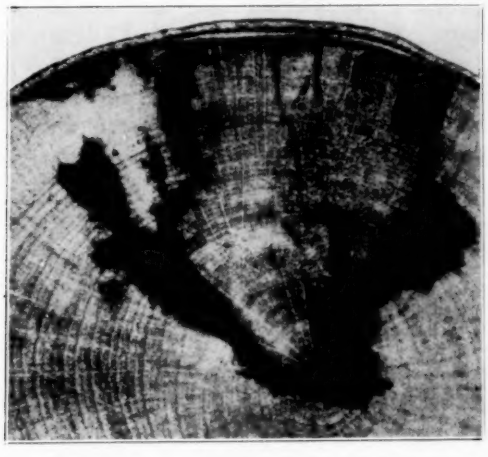

FIG. 5. Antitoxic reaction of a beech trunk to artificial infection by *Stereum purpureum*. Approx. half nat. size. After Münch (8).

fore does not develop the disease. Human medicine possesses an indication of such induced tolerance in typhus carriers.

For instance, if the virus of the ring-spot disease be transferred to healthy tobacco plants by means of rubbing the leaves, typical symptoms appear on these leaves within three days, and the virus spreads through all parts of the plant, with the exception of the growing point. However, the symptoms gradually begin to fade out; those in newly formed leaves and lateral branches become increasingly faint and eventually disappear entirely. The leaves on such plants are merely somewhat darker green and more leathery than on virus-free specimens (9, 10). In the meantime the leaves originally infected have dropped off; the plants have "recovered"; they are clinically cured, but continue to be carriers of the virus, which is still present, unweakened and fully pathogenic to uninfected plants. Only the symptoms have disappeared.

This clinical healing is apparently based on three components:

1. Actual anti-infectional reactions (formation of anti-virus bodies). As a result of these, the virus protein content in recovered leaves is, in general, only 10–20 per cent of that in the originally diseased leaves.

2. A blocking of the remaining 10–20 per cent of the virus proteins. These are in no way blunted, but fully pathogenic to other plants; only in the "healed" plants are they unable to destroy the chloroplasts and to cause necrotic degeneration.

3. A desensitizing of the host organism, *i.e.*, a conversion to tolerance, by virtue of which the host is able to carry the remaining virus (which assimilates a considerable part of the host's native protein) without manifesting symptoms.

When clinically healed carriers are strongly superinfected with the ring-spot virus, they evince no further symptoms; they have thus become tolerant towards even a severe superinfection. This induced tolerance is group-specific against all types of ring-spot virus; the latter can develop undisturbed in the "cured" plants, while retaining full pathogenicity for other plants; only the "recovered" plants ignore, and do not react to them.

If a healthy shoot be grafted on to the stem of a "recovered" tobacco plant, it takes up the virus, but not the acquired tolerance, so that it evinces incipient symptoms of ring-spot which, however, later fade out.

In curly-top of sugar beet however, inoculated to tobacco, not only the virus, but also the induced tolerance entity emigrates to the grafted shoots (19). The "recovered" partner thus induces in the healthy partner another reaction-norm against the virus; a similar induction is found in the organizer-effect in animal transplantations (16).

These six main types of defensive reactions probably do not exhaust the possibilities of active resistance in plants. The aim of this article is merely to describe them shortly with a consistent terminology.

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TEN ADDITIONAL LEAFHOPPER VECTORS OF THE VIRUS CAUSING PIERCE'S DISEASE OF GRAPES

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INTRODUCTION

Three species of leafhoppers, *Draeculacephala minerva* Ball, *Carneocephala fulgida* Nott., and *Neokolla circellata* (Baker), were stated by Hewitt, Frazier, Jacob, and Freitag (4) in 1942 to have the ability to transmit the virus causing Pierce's disease of grapes. Frazier (3) reported these three and, in addition, the following six species as vectors in 1944; *Carneocephala triguttata* Nott., *Helochara delta* Oman, *Neokolla gothica* (Sign.), *N. confluens* (Uhler), *N. heiroglyphica* (Say) and *Cuerna occidentalis* Oman and Beamer.

Hewitt, Houston, Frazier, and Freitag (5) later published experimental data substantiating the fact that *Draeculacephala minerva, Carneocephala fulgida, Neokolla circellata* and *Helochara delta* can transmit the virus, and showed that alfalfa dwarf disease and Pierce's disease of grape are caused by the same virus.

The present paper presents experimental data supporting the fact that the remaining 5 previously reported species, namely, *Carneocephala triguttata, Neokolla gothica, N. confluens, N. heiroglyphica,* and *Cuerna occidentalis* are vectors of the virus, and contains evidence to show that an additional 5 species of leafhoppers, not previously reported, are also able to transmit the virus. They are *Pagaronia triunata* Ball, *P. 13-punctata* Ball, *P. furcata* Oman, *P. confusa* Oman, and *Friscanus friscanus* (Ball).[3]

METHODS

All work herein reported was carried out at Berkeley under glasshouse conditions. Naturally and experimentally infected alfalfa and grape plants were used as sources of virus for the leafhoppers. Healthy California Common variety alfalfa plants and grapevines of the varieties Emperor, Thompson Seedless, Ribier, and Palomino propagated from indexed cuttings were used as test plants. The numbers of tests made of the different species of leafhoppers were determined in large measure by their availability. The numbers of insects used per test were usually from 5 to 15, but the great majority of tests was made with 10. It was not found possible to effectively rear sufficient populations of any of the species for experimental work, so it was necessary to collect all insects in the field. Because of limited distri-

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[3] Credit is due to Dr. R. H. Beamer for the determinations of *Carneocephala triguttata* and *Cuerna occidentalis;* to Dr. D. M. Delong for determining *Neokolla gothica, N. confluens, N. hieroglyphica,* and *Friscanus friscanus;* and to Dr. R. I. Sailer for determining *Pagaronia triunata, P. furcata* and *P. 13-punctata.*

bution and relative scarcity of some species often only small numbers could thus be obtained. With the genera *Pagaronia* and *Friscanus*, grape and alfalfa proved to be rather unfavorable hosts inasmuch as a relatively high mortality resulted, and this again limited the number of tests.

TRANSMISSION OF VIRUS

The results of transmission trials carried out with 10 species of leafhoppers are in table 1. Under the conditions of these experiments a considerable range was evidenced among the various species in their relative ability to transmit the virus from diseased to healthy alfalfa and grape plants. The most efficient group of vectors included the species of the genera *Carneocephala*, *Neokolla*, and *Cuerna* which infected from 42 to 78 per cent of the total number of plants inoculated. Grape or alfalfa were relatively favorable host plants for the species belonging to these 3 genera,

TABLE 1.—*Transmission by 10 species of leafhoppers of virus from diseased to healthy grape and alfalfa plants*

Species of leafhoppers	From diseased grape		From diseased alfalfa		Percentage of total infected
	To grape	To alfalfa	To grape	To alfalfa	
Carneocephala triguttata	3/4[a]	0/1	3/7	4/12	42
Neokolla gothica	13/16	3/5	8/15	4/21	49
N. confluens	7/7	1/3	4/6	2/2	78
N. heiroglyphica	2/2	0/0	2/4	1/4	50
Cuerna occidentalis	9/10	2/3	3/3	2/4	76
Pagaronia triunata	0/7	0/3	0/3	3/10	13
P. 13-punctata	0/0	0/0	0/0	1/3	33
P. furcata	9/35	2/13	5/25	5/39	19
P. confusa	2/23	1/16	3/14	4/16	14
Friscanus friscanus	0/0	0/0	3/3	3/25	21

[a] The numerator indicates the number of plants infected; the denominator indicates the number of plants inoculated.

as judged by the longevity of the insects when caged on them. The species of the genera *Pagaronia* and *Friscanus*, however, survived relatively poorly on grape or alfalfa, and with these species the percentage of transmission of virus, as shown in table 1, was usually low, ranging from 13 to 33. In the cases of *P. triunata* which yielded but 3 infections of 23 tests and *P. 13-punctata* which was tested only 3 times and caused only 1 infection it is evident that, if considered separately, the number of cases of infection is too low ordinarily to be acceptable as proof of a species' ability to transmit a virus. These 2 species are listed in this instance, however, because 2 other species in the same genus yielded a comparably low but consistent number of infections over a much larger series of tests and it seems safe to assume that the same would hold true should *P. triunata* and *P. 13-punctata* be subjected to further testing.

None of the 10 species listed in table 1 appears to be of any importance under field conditions in the spread of the virus to either alfalfa or grapevines since they are only rarely found in vineyards or alfalfa fields.

PHYLOGENETIC RELATIONSHIP OF PIERCE'S DISEASE VECTORS

Fourteen species of leafhoppers in 7 genera, listed earlier in this paper, have been shown to be capable of transmitting the virus of Pierce's disease. They are all contained in the leafhopper (Homoptera-Cicadellidae) subfamily Tettigoniellinae (2) (= Cicadellinae (6) = Amblycephalinae (1)), members of which are commonly known as sharpshooters.

Early in the search for a vector of the virus a valuable clue was provided in a vineyard in which insect-electrocuting light traps were being operated as described in a previous publication (5). Under certain of the traps very definite accumulations of diseased vines had occurred (5, fig. 1) and under these same traps marked concentrations of populations of *Draeculacephala minerva* and *Carneocephala fulgida* were noted. Other factors also served to strengthen the suspicion that either or both insects might be the virus carrier. Subsequent tests proved both to be vectors.

Neokolla circellata became strongly suspected of being a vector because of its abundance on wild and cultivated grapevines in localities where Pierce's disease was prevalent and neither of the known vectors, *Draeculacephala minerva* or *Carneocephala fulgida*, was present in sufficient numbers to satisfactorily account for the amount of disease.

When *Neokolla circellata* was demonstrated to be a vector of Pierce's disease virus it became the third species belonging to the subfamily Tettigoniellinae known to have the ability to transmit the virus, and the other species of the subfamily were then suspected as possible vectors. Consequently an effort was made to test as many species as conveniently possible in order to explore the possibility that the ability to transmit the virus might be common to all species of the subfamily. That the ability to transmit the virus can definitely be correlated with a phylogenetic relationship of the vectors within subfamily limits is evidenced by the fact that every species of the subfamily Tettigoniellinae thus far tested has the ability to transmit the virus. During the course of the experiments more than 50 species of leafhoppers contained in subfamilies other than Tettigoniellinae were tested and not one proved to be a vector.

It is not implied that the ability to transmit the virus of Pierce's disease is necessarily specific or limited only to species within the Tettigoniellinae. It is quite possible that species in other subfamilies or families may have that ability. Nor is it implied that every species of the Tettigoniellinae must necessarily be a vector, although the evidence strongly suggests that the ability to transmit the virus is a group characteristic and each species should be suspected of the ability until proven otherwise.

SUMMARY

Ten additional species of leafhoppers are demonstrated to be vectors of the virus of Pierce's disease of grapes. These are *Carneocephala triguttata, Neokolla gothica, N. confluens, N. heiroglyphica, Cuerna occidentalis, Pagaronia triunata, P. 13-punctata, P. furcata, P. confusa,* and *Friscanus friscanus.*

None of the 10 species appears to be of any importance under field conditions in the spread of the virus to alfalfa or grapevines.

All 14 vectors of Pierce's-disease virus thus far demonstrated are contained in the leafhopper subfamily Tettigoniellinae of which every species thus far tested has transmitted the virus. More than 50 species of leafhoppers contained in other subfamilies were tested and not one proved to be a vector. The evidence indicates that the ability to transmit the virus can be correlated with a phylogenetic relationship of the vectors within the Tettigoniellinae.

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EFFECT OF SOIL TEMPERATURE ON THE DEVELOPMENT OF RHIZOCTONIA ROOT CANKER OF ALFALFA[1]

OLIVER F. SMITH[2]

(Accepted for publication April 10, 1946)

It is well known that soil temperature influences the development of certain plant diseases caused by *Rhizoctonia solani* Kühn. In some cases (1, 2, 3, and 8) the disease develops best at high soil temperatures; whereas, in other cases (4, 5, 6) it develops best at low soil temperatures. In a previous publication (7) it was noted that Rhizoctonia root canker of alfalfa is seasonal in its development and is closely correlated with warm soil temperatures. Cankers develop most abundantly during June, July, August, and September when soil temperatures, at 3 inches below the surface, are above 20° C., but no cankers develop during the winter months when soil temperatures, at this same depth, may be as low as 5° to 10° C. This seasonal development of the disease suggests that soil temperature is an important factor influencing its development.

This paper reports results from experiments to determine the influence of temperature on the growth of *Rhizoctonia solani* and the effect of soil temperature on the development of Rhizoctonia root canker of alfalfa.

EFFECT OF TEMPERATURE ON GROWTH OF THE ORGANISM

The effect of temperature on growth of the organism was determined by growing the organism, in triplicate, in Petri dishes on potato-dextrose agar at constant temperatures of 5°, 10°, 15°, 20°, 25°, 30°, 35°, and 40° C.[3] A small amount of mycelium was placed at the center of each dish and the diameters of the colonies determined at the end of 3 days (Fig. 1). The organism grew most rapidly at 25° and 30° C. No growth occurred at 5°, 10°, and 40° C. during the 3-day period. In another experiment, a small amount of growth occurred at 10° C. after 11 days.

TABLE 1.—*Number of cankers per main tap root of alfalfa plants inoculated with isolate 102 of Rhizoctonia solani from alfalfa and grown at different soil temperatures for 50 days*

No. of roots	No. of cankers per root after 50 days at soil temperatures of:					
	15° C.	20° C.	25° C.	30° C.	35° C.	40° C.
25	0	13.3	21.3	29.4	6.1	0

[1] Cooperative investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Nevada Agricultural Experiment Station.

[2] Associate Pathologist, Division of Forage Crops and Diseases, U. S. Department of Agriculture.

[3] Relation of temperature to growth on potato-dextrose agar was determined by H. W. Johnson, Senior Pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

EFFECT OF SOIL TEMPERATURE ON DISEASE DEVELOPMENT

For determining the effect of soil temperature on disease development, 4-months-old alfalfa plants growing in galvanized iron cans (25 plants per can) were inoculated with culture 102 of *Rhizoctonia solani* and maintained at soil temperatures of 15°, 20°, 25°, 30°, 35°, and 40° C. for 50 days. Soil temperatures were maintained by thermostatically controlled water baths. Inoculation technique was the same as described elsewhere (7). At the end of 50 days the roots were removed from the soil and the number of cankers on the main tap root was determined (Table 1). There were no root cankers at soil temperatures of 15° and 40° C. and an average of 29.4

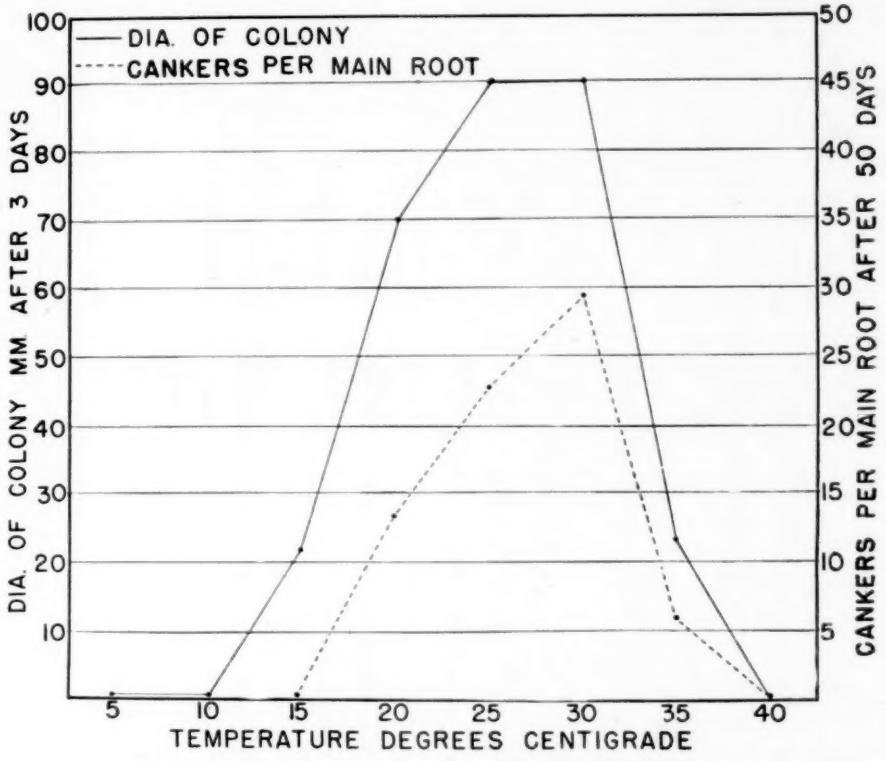

FIG. 1. Effect of temperature on growth of *Rhizoctonia solani* on potato-dextrose agar and of soil temperature on the development of Rhizoctonia root cankers on alfalfa.

cankers on each tap root at a soil temperature of 30° C. Figure 2 shows the degree of root canker development at the different soil temperatures. From the figure it appears there are small cankers on the roots grown at a soil temperature of 40° C. but control plants grown at this same soil temperature, and not inoculated, had small cankers similar to those on the inoculated plants and they are considered to be a result of the soil temperature being too warm for healthy alfalfa plant growth. That a soil temperature of 40° C. was too warm for the plants was also evidenced by short and unhealthy-appearing top growth of the alfalfa.

As previously stated, root cankers, as observed in the field, develop during the warm part of the season. At Bard, California, in fields cropped

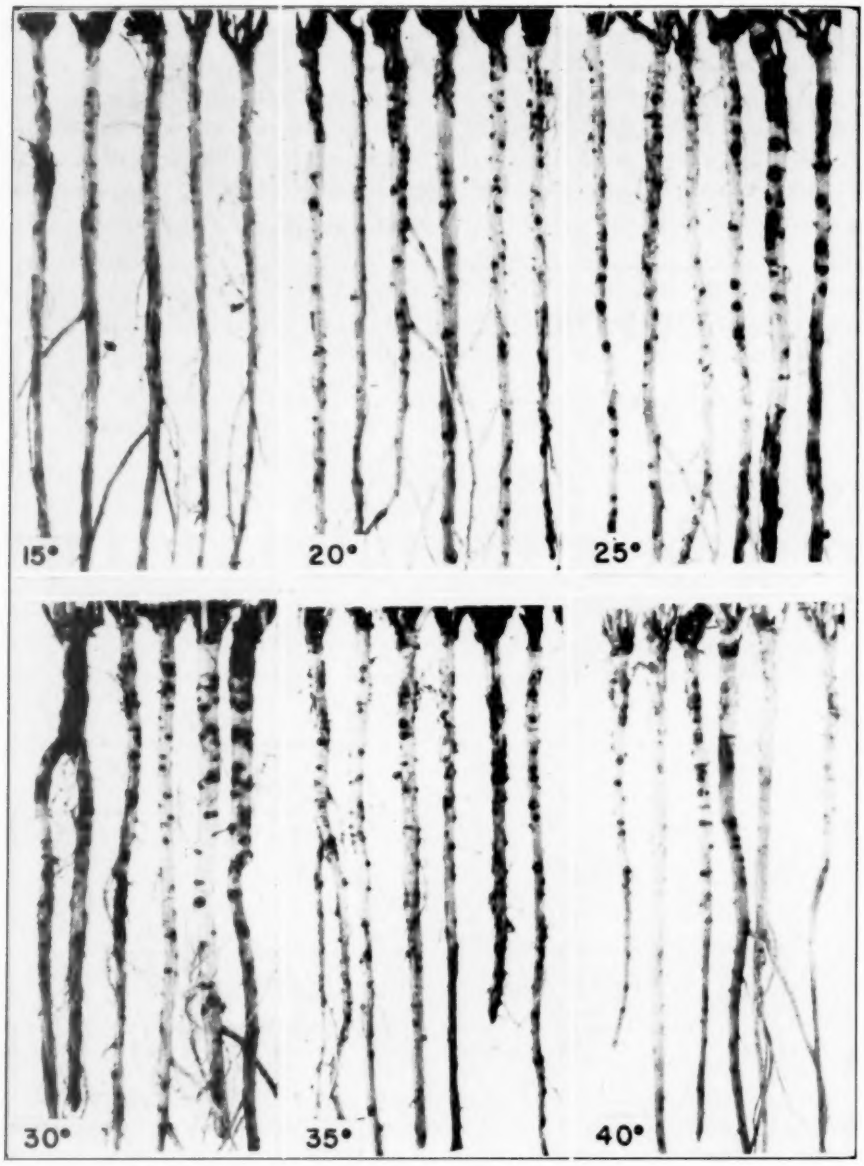

FIG. 2. Effect of soil temperature on the development of Rhizoctonia root canker of alfalfa. Roots were inoculated with *Rhizoctonia solani* and grown at the soil temperatures indicated for 50 days.

with alfalfa, the soil, at 3 inches below the surface, does not warm to 20° C. before about the middle of April and cools below 20° C. about the middle

of October (Fig. 3).[4] During this period soil temperatures are above 20° C. and are favorable to disease development, as determined by controlled experiments (Fig. 1). Soil temperature is the main factor which influences the development of alfalfa Rhizoctonia root canker.

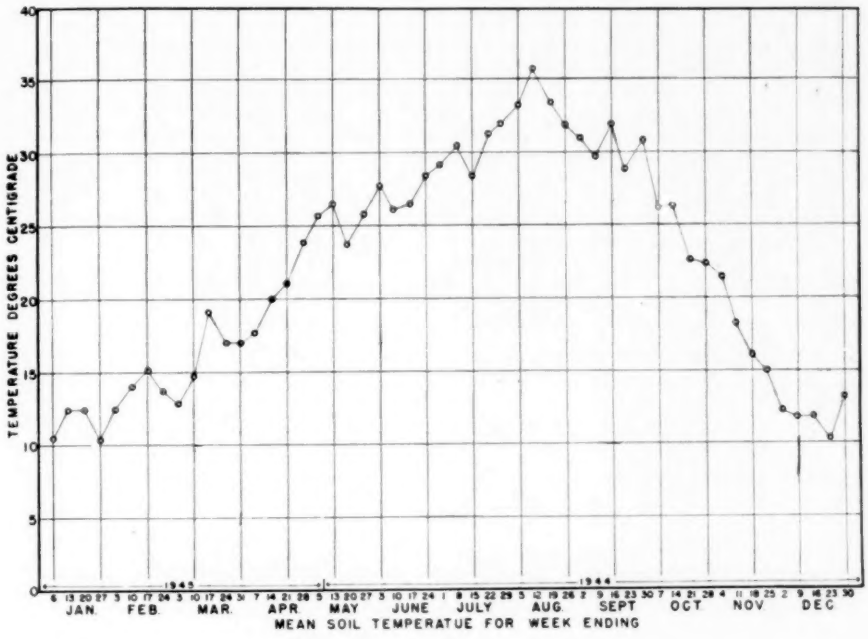

FIG. 3. Mean weekly soil temperature 3 inches below the surface at Bard, California, from May 7, 1944, to May 5, 1945.

SUMMARY

Optimum temperature for growth of *Rhizoctonia solani* on potato-dextrose agar was about 25–30° C. Optimum soil temperature for development of Rhizoctonia root canker of alfalfa was about 25–30° C. Very few cankers developed at a soil temperature below 20° or above 35° C. in 50 days.

In the field, very few root cankers develop before the soil warms to 20° C. or higher in the spring, and cankers cease to develop in the fall after the soil cools to approximately 20° C. or lower.

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[4] Records of soil temperatures at Bard, California, were supplied by E. G. Noble, Agronomist, Division of Irrigation Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

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A COMPARISON OF THE EXPERIMENTAL HOST RANGES OF TOBACCO-ETCH AND TOBACCO-MOSAIC VIRUSES

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Tests of the experimental host ranges of tobacco-etch virus (*Marmor erodens* H.) and tobacco-mosaic virus (*M. tabaci* H.) have been conducted over several years. The present paper summarizes briefly what has been learned from them and records the behavior of all species of plants that were inoculated during the experiments.

REVIEW OF LITERATURE

Tobacco-etch and tobacco-mosaic viruses have been reported as infecting only a few plant species in nature. *Nicotiana tabacum* L., *Lycopersicon esculentum* Mill., *Capsicum frutescens* L., *Physalis heterophylla* Nees, and *Petunia hybrida* Vilm. (12) have been found infected with each of the two viruses. In addition, tobacco-etch virus (under the name of "virus of mosaic C") has been recovered from naturally infected plants of *Datura stramonium* L., its first recognized host (4), and *Physalis subglabrata* Mackenzie and Bush (11); tobacco-mosaic virus has been recovered from naturally infected plants of *Solanum carolinense* L. (12). All of these species are of the family Solanaceae. A specialized strain of tobacco-mosaic virus has been reported as infecting *Plantago lanceolata* L. and *P. major* L. in an additional family, the Plantaginaceae (9).

Experimental studies have demonstrated a much wider potential range of hosts for both viruses. It has long been known that the typical strain of tobacco-mosaic virus is able to infect a large proportion of all inoculated species of solanaceous plants; indeed among many such species tested experimentally in the past only one, *Physalis viscosa* L., has been shown to be naturally immune (6). In this paper, evidence is presented that at least one other solanaceous species, *Cyphomandra betacea* Sendt. (See table 1), is immune from infection by rubbing inoculation, but it is evident that a great majority of solanaceous plants are susceptible to infection under appropriate circumstances. Fernow (4) was the first to show experimentally that tobacco-mosaic virus might cause disease in a representative of another family; he described its effects in *Martynia louisiana* Mill., of the Martyniaceae. Price (14) found some varieties of the common bean, *Phaseolus vulgaris* L., to be susceptible on inoculation and to be suitable substitutes for *Nicotiana glutinosa* L. of the Solanaceae in estimating concentrations of this virus. This added another, the Leguminosae, to the two families already known to include susceptible species. Grant (5) showed that potential hosts of this virus are in reality widely scattered among herbaceous dicotyledons, reporting 29 susceptible species in 14 families other than the Solanaceae. In a subsequent study by the writer, the ability of

plant species in many families to support the multiplication of tobacco-mosaic virus in their tissues, after its introduction by mechanical inoculation, was confirmed and this susceptibility was found to be correlated with taxonomic affiliations of the experimental hosts (7). One additional extension of the experimental host range of tobacco-mosaic virus has been provided by Severin (15), who found recently that guayule (*Parthenium argentatum* A. Gray of the family Compositae) developed small necrotic areas at the site of inoculation and supported a multiplication of virus locally.

Not only does the experimental host range of tobacco-mosaic virus far outrun the bounds of the Solanaceae, but that of tobacco-etch virus also transcends those limits. In the course of a previously reported investigation (10), aimed at securing a host that would show conspicuous primary lesions after inoculation with etch virus, many additional species were found susceptible. These species were not, however, specifically listed in the publication cited. In a brief abstract (11) it was reported that all species found capable of supporting the increase of tobacco-etch virus seemed also capable of supporting increase of tobacco-mosaic virus if inoculated but that the converse was not true. Complete data are given in the present paper.

MATERIALS AND METHODS

The strain of tobacco-mosaic virus used for the tests recorded in this paper was the green-mottling, distorting strain (var. *vulgare* H.) that seems to be rather typical of tobacco-adapted isolates; the strain of tobacco-etch virus was that known as the severe-etch strain (var. *severum* H.), an isolate of remarkable constancy originally obtained from E. M. Johnson, who first described it (12). This severe-etch strain represents a naturally-occurring variant of the virus originally reported as the cause of a disease called "etched" by Valleau and Johnson (17) and later referred to as etch (12). Use of strains other than these two would have produced additional symptoms and otherwise modified the data here presented but could not be included without greatly altering the scope of the investigation.

Inoculum was prepared differently for the two viruses because of differences in infectivity and stability. A once-frozen and thawed sample of a 1:50 aqueous dilution of juice expressed from diseased tobacco plants was used as the tobacco-mosaic inoculum, to avoid retention of measurable amounts of virus on the surface of inoculated but not infected leaves. Freshly expressed whole juice from tobacco plants, usually obtained 10 days after infection, was used for severe-etch inoculum, except in a few cases in which similar undiluted juice expressed from infected plants of the species *Nicotiana glutinosa* was substituted for it.

All inoculations were made by rubbing the upper surfaces of leaves with pads of cheesecloth moistened with the above described inocula. Additional opportunity for entrance of virus was provided by 100 small pin punctures made through the moistened inoculated leaves to mark the areas of inoculation in each plant.

EXPERIMENTAL RESULTS

Evidence that the two viruses used in the present investigation are not closely related was obtained by observing their failure to protect against each other in suitable hosts. In figure 1, A is represented a leaf of *Physalis peruviana* Mill., mottled as a result of systemic infection by tobacco-mosaic virus yet bearing necrotic primary lesions caused by subsequent inoculation of the affected leaf with severe-etch virus; the necrotic lesions occurred both

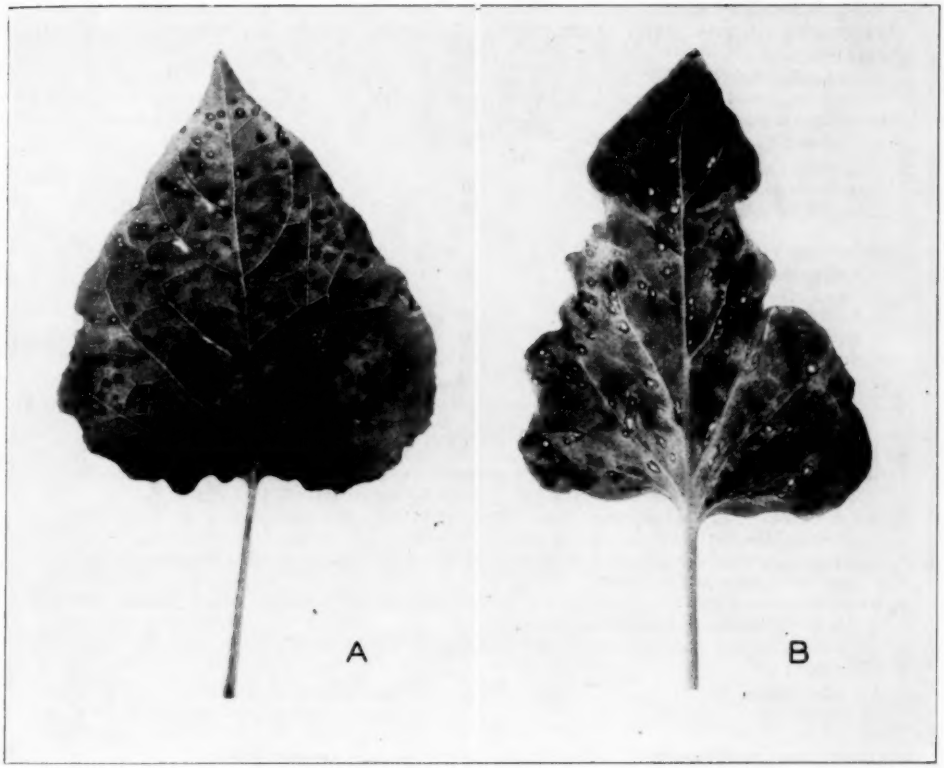

FIG. 1. A. Leaf of *Physalis peruviana*, mottled as a result of systemic infection by tobacco-mosaic virus yet bearing numerous necrotic primary lesions as a result of subsequent inoculation with severe-etch virus. Most of the necrotic lesions occurred in chlorotic areas of the leaf, because green areas of the mottling pattern were relatively small. B. Leaf of *Nicotiana glutinosa*, similarly mottled as a result of systemic infection by severe-etch virus yet bearing necrotic primary lesions as a result of subsequent inoculation with tobacco-mosaic virus. (Photograph by J. A. Carlile.)

in such green areas as were present and in the more extensive chlorotic areas of the mottled leaf. In figure 1, B is represented a leaf of *Nicotiana glutinosa*, mottled and distorted as a result of systemic infection by severe-etch virus, yet bearing necrotic primary lesions formed as a result of subsequent inoculation with tobacco-mosaic virus; here also the necrotic lesions occurred both in the green blister-like areas and in the more extensive chlorotic areas of the mottled leaf. This failure of protection against formation

TABLE 1.—*Results of inoculations made to compare experimental host ranges of tobacco-etch and tobacco-mosaic viruses*

Family and species	Results of inoculation[a, b]	
	With tobacco-etch virus	With tobacco-mosaic virus
AIZOACEAE		
Mesembryanthemum crystallinum L.	0	1
M. lineare Thunb.	0	4 (L, V–O; S, V–CM)
Mollugo verticillata L.	1	1
Tetragonia expansa Murr.	3 (L, V–CSp; S, Va–O)	3 (L, V–CSp–Ny; S, Va–O)
AMARANTHACEAE		
Amaranthus tricolor L.	0	1
Celosia argentea L.	3 (L, V–CSp; S, Va–O)	4 (L, V–CSp; S, V–CSp–CM)
Gomphrena haageana Klotzsch	0	0
BALSAMINACEAE		
Impatiens balsamina L.	0	0
I. holstii Engler and Warb.	0	3 (L, V–CSp–NRiCo; S, Va–O)
CAMPANULACEAE		
Campanula carpatica Jacq.	0	0
C. drabifolia Sibth. and Sm.	2	2
C. lactiflora Bieb.	0	0
C. medium L.	0	0
C. pyramidalis L.	0	0
C. rotundifolia L.	0	0

[a] Numbers have the following significance:

0, no increase of virus and no manifestation of disease (tested species insusceptible).

1, increase of virus locally but neither systemic spread of virus nor production of an obvious disease (tested species susceptible to localized masked disease).

2, virus detected in leaves other than those originally inoculated but no production of obvious disease (tested species susceptible to systemic masked disease).

3, local disease manifestations but no spread of virus (tested species susceptible to localized but obvious disease).

4, systemic spread of virus and obvious manifestations of disease (tested species susceptible to systemic and obvious disease).

[b] Abbreviations used within explanatory parentheses in the table have the following significance:

Ab, abscission
Bl, black
Br, brown
C, chlorosis or chlorotic
Ch, chlorophyll-retention patterns
Co, concentric patterns
Col, collapse
D, death of plant
Dif, diffuse
Dis, distortion or distorted
E, etched patterns
En, enations
Ex, extreme
Fcd, flower color deepened
G, green or greenish
K, klendusic (often escaping infection)
L, locally
Le, leaf or leaves
M, mottling
Ma, masking
Mv, midvein
N, necrosis or necrotic
Ny, necrosis with surrounding yellowing
O, no manifestation of disease observed
Pc, pale center
Pe, petiole or petioles
Pu, puckered
R, red
Ri, ring-like spots
S, systemically
Se, segments of rings
Sp, spots
St, stem or stems
Str, streak
Stu, stunting
V, virus recoverable
Va, virus absent
Ve, veinclearing
W, white
Wi, wilting
Y, yellowing or yellow
Yo, young

TABLE 1.—(*Continued*)

Family and species	Results of inoculation[a, b]	
	With tobacco-etch virus	With tobacco-mosaic virus
CAPPARIDACEAE		
Cleome spinosa L.	0	1
Polanisia trachysperma Torr. and Gray	0	2
CARICACEAE		
Carica papaya L.	0	3 (L, V–CSp; S, Va–O)
CARYOPHYLLACEAE		
Dianthus barbatus L.	0	0
D. chinensis L.	0	0
Gypsophila elegans Bieb.	1	1
Lychnis coeli-rosa (L.) Desr.	0	1
Silene anglica L.	4 (L, V–NRi; S, V–NRi–E)	1
S. pendula L.	0	1
Stellaria media (L.) Cyrill.	0	1
CHENOPODIACEAE		
Beta vulgaris L.	0	3 (L, V–CSpRRi; S, Va–O)
Chenopodium album L.	0	3 (L, V–CSp–RNSp; S, Va–O)
C. ambrosioides L.	0	3 (L, V–CSp–NSp; S, Va–O)
C. glaucum L.	0	3 (L, V–CSp; S, Va–O)
C. rubrum L.	0	3 (L, V–CSp–LeAb; S, Va–O)
Kochia scoparia Schrad.	0	1
Spinacia oleracea L.	0	1
COMPOSITAE		
Ageratum conyzoides L.	0	0
Ambrosia trifida L.	0	1
Arctium lappa L.	0	1
Bellis perennis L.	0	3 (L, V–CSp; S, Va–O)
Bidens frondosa L.	0	1
Brachycome iberidifolia Benth.	4 (L, Va–O; S, V–CM)	2
Calendula officinalis L.	0	3 (L, V–CSp; S, Va–O)
Callistephus chinensis Nees	3 (L, V–BrNRi; S, Va–O)	1
Centaurea americana Nutt.	0	1
C. montana L.	0	1
C. moschata L.	0	0
Charicis heterophylla Cass.	4 (L, V–O; S, V–CSp)	1
Chrysanthemum leucanthemum L.	0	0
Cichorium endivia L.	0	3 (L, V–CCoRi; S, Va–O)
Coreopsis grandiflora Nutt.	0	3 (L, V–CSp; S, Va–O)
Dahlia pinnata Cav.	0	0
Dimorphotheca aurantiaca DC.	4 (L, V–BrNSp; S, V–BrNSp–CM–NStStr)	2
Emilia flammea Cass.	0	4 (L, V–O; S, V–CM–CSp)
Erigeron annuus (L.) Pers.	0	1
E. speciosus DC.	0	0
Eupatorium lasseauxii Carr.	1	1
Gaillardia aristata Pursh	0	0
Galinsoga parviflora Cav.	0	1
Gamolepis tagetes DC.	2	1
Helianthus annuus L.	3 (L, V–NRi; S, Va–O)	1
H. tuberosus L.	0	0
Helichrysum bracteatum Andr.	0	3 (V–CSp; S, Va–O)

TABLE 1.—(*Continued*)

Family and species	Results of inoculation[a, b]	
	With tobacco-etch virus	With tobacco-mosaic virus
Heliopsis scabra Dun.	0	0
Helipterum humboltianum DC.	1	1
Leptosyne maritima (Hook. f.) A. Gray	0	1
Rudbeckia hirta L.	0	3 (L, V–CSp; S, Va–O)
Sanvitalia procumbens Lam.	0	1
Solidago rugosa Mill.	0	0
Tagetes erecta L.	0	2
T. patula L.	0	1
T. signata Bartl.	0	1
Taraxacum officinale Weber	0	0
Thelesperma hybridum Voss	0	0
Zinnia elegans Jacq.	4 (L, V–O; S, V–CM)	1
Z. haageana Regel	0	2
CONVOLVULACEAE		
Calonyction aculeatum House	0	1
Ipomoea batatas Lam.	0	0
I. nil Roth	0	1
I. setosa Ker	0	0
I. tricolor Cav.	0	1
CRUCIFERAE		
Barbarea vulgaris R. Br.	0	0
Brassica napobrassica Mill.	0	1
B. oleracea L.	0	0
B. pekinensis Rupr.	0	0
Capsella bursa-pastoris (L.) Medic.	0	1
Cheiranthus allionii Hort.	0	1
C. cheiri L.	0	1
Iberis gibraltarica L.	0	1
I. sempervirens L.	0	0
I. umbellata L.	0	1
Lobularia maritima Desv.	0	1
Lunaria annua L.	0	4 (L, V–O; S, V–LeDis) or 2
Mathiola bicornis DC.	0	1
M. incana R. Br.	0	0
Raphanus raphanistrum L.	0	0
R. sativus L.	0	0
GERANIACEAE		
Geranium carolinianum L.	0	1
Pelargonium hortorum Bailey	0	0
P. odoratissimum Ait.	0	0
GESNERIACEAE		
Saintpaulia ionantha Wendl.	0	0
Sinningia speciosa Benth. and Hook.	0	1
HYDROPHYLLACEAE		
Emmenanthe penduliflora Benth.	4 (L, V–BrNSp; S, V–BrNSp–D)	2
Nemophila insignis Benth.	1	1
N. maculata Benth.	1	3 (L, V–BrNSpRi; S, Va–O)

TABLE 1.—(*Continued*)

Family and species	Results of inoculation[a, b]	
	With tobacco-etch virus	With tobacco-mosaic virus
Phacelia campanularia Gray	4 (L, V–O; S, V–Ve–CM)	4 (L, V–O; S, V–CSp)
P. ciliata Benth.	2	2
P. grandiflora (Benth.) Gray	4 (L, V–CSp; S, V–CM–Ma)	4 (L, V–O; S, V–CVe–CM)
P. viscida (Benth.) Torr.	4 (L, V–O; S, V–Ve–N–NStStr)	4 (L, V–O; S, V–CM)
P. whitlavia Gray	4 (L, Va–O; S, V–NSe)	4 (L, V–O; S, V–CM)
LABIATAE		
Coleus blumei Benth.	0	1
Lamium amplexicaule L.	4 (L, V–O; S, V–CM–Ma)	2
Lycopus rubellus Moench	0	1
Marrubium vulgare L.	0	1
Melissa officinalis L.	0	1
Mentha spicata L.	0	1
Nepeta cataria L.	0	1
N. hederacea (L.) Trev.	0	0
N. mussini Spreng.	0	1
Prunella vulgaris L.	0	1
Salvia azurea Lam.	0	1
S. farinacea Benth.	0	1
S. patens Cav.	0	1
S. splendens Ker	0	1
LEGUMINOSAE		
Dolichos lablab L.	0	0
Glycine max (L.) Merr.	0	0
Lens esculenta Moench	0	0
Lupinus hartwegii Lindl.	0	0
Medicago sativa L.	0	0
Phaseolus coccineus L.	0	0
P. aureus Roxb.	0	0
P. limensis Macf.	0	0
P. vulgaris L. (according to variety)	0	3 (L, V–RNSp; S, Va–O) or 0
Pisum sativum L.	0	0
Trifolium incarnatum L.	0	0
T. pratense L.	0	0
T. repens L.	0	0
Vicia faba L.	0	0
V. sativa L.	0	0
Vigna sinensis (L.) Endl.	0	0
LOBELIACEAE		
Lobelia erinus L.	0	1
L. gracilis Andr.	4 (L, V–O; S, V–DifCM)	1
L. inflata L.	4 (L, V–O; S, V–CM)	1
L. tenuior R. Br.	4 (L, V–O; S, V–C–Wi–N)	1
MARTYNIACEAE		
Proboscidea louisiana (Mill.) Woot. and Stand.	4 (L, V–O; S, V–Ve–CM)	4 (L, V–O; S, V–CM)
NOLANACEAE		
Nolana lanceolata Miers	4 (L, V–O; S, V–CVe–N–Stu–CM–Dis–LePu)	4 (L, V–O; S, V–CM)
PAPAVERACEAE		
Chelidonium majus L.	0	3 (L, V–DifC; S, Va–O)
Hunnemannia fumariaefolia Sweet	0	0
Papaver nudicaule L.	0	3 (L, V–C–N; S, Va–O)
P. orientale L.	0	0

TABLE 1.—(*Continued*)

Family and species	Results of inoculation[a, b]	
	With tobacco-etch virus	With tobacco-mosaic virus
PHYTOLACCACEAE		
Phytolacca decandra L.	0	3 (L, V–CRi–RRi; S, Va–O)
PLANTAGINACEAE		
Plantago lanceolata L.	0	1
P. major L.	0	3 (L, V–NBrRi; S, Va–O)
P. rugelii Dene.	0	3 (L, V–CSp; S, Va–O)
POLEMONIACEAE		
Cobaea scandens Cav.	0	0
Gilia capitata Dougl.	0	1
G. liniflora Benth.	0	2
Phlox drummondii Hook.	0	4 (L, V–CSp–CCoRi; S, V–CSp)
PORTULACACEAE		
Portulaca oleracea L.	3 (L, V–LeWiAb; S, Va–O)	1
PRIMULACEAE		
Anagallis arvensis L.	0	1
Primula malacoides Franch.	2	1
P. obconica Hance	0	1
SCROPHULARIACEAE		
Antirrhinum majus L.	0	1
Collinsia bicolor Benth.	3 (L, V–CSp; S, Va–O)	4 (L, V–O; S, V–CM)
Cymbalaria muralis Gaertn., Mey. and Scherb.	1	1
Digitalis purpurea L.	0	4 (L, V–NSe; S, V–O)
Linaria maroccana Hook. f.	2	1
L. vulgaris Hill	0	1
Mimulus moschatus Dougl.	0	1
M. tigrinus Hort.	0	1
Nemesia strumosa Benth.	0	2
Penstemon grandiflorus Nutt.	1	1
Torenia fournieri Lind.	1	2
Verbascum phoeniceum L.	0	1
Veronica longifolia L.	0	1
V. officinalis L.	0	1
V. peregrina L.	0	1
Zaluzianskya villosa Schmidt	4 (L, V–CSp; S, V–O)	2
SOLANACEAE		
Browallia americana L.	0	3 (L, V–CSp–CRi–Ch; S, Va–O)
B. speciosa Hook.	4 (L, V–O; S, V–CM–Stu)	4 (L, V–CSp; S, V–C–CM) or 3 (L, V–BrNSpPe–LeAb; S, Va–O)
Capsicum frutescens L.	4 (L, V–O; S, V–CM)	4 (L, V–CSp–Ch; S, V–C–Stu–CM–Ch) or 4 (L, V–CSp–NSp–LeAb; S, V–CSp–NSp) or 3 (L, V–NSp–LeAb; S, Va–O) or 3 (L, V–CSp–NSp–LeAb; S, Va–O)
Cyphomandra betacea Sendt.	0	0
Datura stramonium L.	4 (L, V–O; S, V–CM–Dis)	3 (L, BrNSpPe; S, Va–O)
Lycium chinense Mill.	3 (L, V–DifCSp or BrNSp; S, Va–O)	3 (L, V–DifCSp; S, Va–O)
Lycopersicon chilense Dun.	0	4 (L, V–O; S, V–CM–K)

TABLE 1.—(*Continued*)

Family and species	Results of inoculation[a, b]	
	With tobacco-etch virus	With tobacco-mosaic virus
L. esculentum Mill.	4 (L, V–O; S, V–CM)	4 (L, V–O; S, V–CM–Dis)
L. hirsutum Dun.	2	2
L. peruvianum (L.) Mill.	4 (L, V–O; S, V–CSp)	1
L. pimpinellifolium (Jusl.) Mill.	4 (L, V–O; S, V–CM)	4 (L, V–O; S, V–CM) or 2
Nicandra physalodes (L.) Pers.	4 (L, V–O; S, V–CM–CSp)	4 (L, V–O or CSp or Ch; S, V–CSp or Ch or CM–Fed)
Nicotiana acuminata (Grah.) Hook.	4 (L, V–C; S, V–CVe–C–Stu–CM)	4 (L, V–BrNSp; S, V–N–StStr) or 3 (L, V–BrNSp; S, Va–O)
N. alata Link and Otto	4 (L, V–O; S, V–Ve–CM–E)	4 (L, V–BlBrNSpPe; S, V–N or L, V–CSp; S, V–CM)
N. bigelovii S. Wats.	4 (L, V–O; S, V–C–Pu–Stu–LeDis–CM)	4 (L, V–CSp or O; S, V–C–Stu–CM or D)
N. bonariensis Lehm.	4 (L, V–DifCSp; S, V–CVe–DifCSp)	4 (L, V–CSp; S, V–CVe–CM–Dis)
N. caudigera Phil.	4 (L, V–Y–N; S, V–CM–Stu)	4 (L, V–CNSp; S, V–C–Pu–Stu–CM–Dis)
N. clevelandii A. Gray	4 (L, V–O; S, V–Stu–CVe–CM–E)	4 (L, V–C; S, V–YoLePu–C–CM)
N. digluta Clausen and Goodspeed	4 (L, V–O; S, V–Ve–CM–Dis)	3 (L, V–BrNSpPe; S, Va–O)
N. glauca Grah.	1	4 (L, V–CSp or O; S, V–CM) or 3 (L, V–CSp; S, Va–O) or 2 or 1
N. glutinosa L.	4 (L, V–CSp; S, V–CM–E)	4 (L, V–BrNSpPe; S, V–NSp) or 3 (L, V–BrNSpPe; S, Va–O)
N. langsdorffii Weinm.	4 (L, V–O; S, V–Ve–GCM)	4 (L, V–BlNSpPe; S, V–N–StStr)
N. longiflora Cav.	4 (L, V–O; S, V–Ve–GCM)	4 (L, V–O; S, V–Stu–CM–Dis)
N. nudicaulis S. Wats.	4 (L, V–O; S, V–CM–Dis)	4 (L, V–CSp; S, V–C–Stu)
N. otophora Griseb.	0	4 (L, V–O; S, V–CM–Stu)
N. palmeri A. Gray	0	4 (L, V–C; S, V–CVe–LeYPu–CM)
N. paniculata L.	4 (L, V–CSp; S, V–CRi)	4 (L, V–CSp; S, V–CVe–CM–En)
N. plumbaginifolia Viv.	4 (L, V–O; S, V–CVe–CM)	4 (L, V–O; S, V–CVe–C–CM–Dis)
N. raimondii Macbride	0	4 (L, V–O; S, V–CSp–CM) or 2
N. repanda Willd.	4 (L, V–CSp or NSe; S, V–CM–CSp)	4 (L, V–BrNSpPe; S, V–N–D)
N. rustica L.	4 (L, V–O; S, V–Ve–CM–Dis)	4 (L, V–BrNSpPe–Ny; S, V–NSp)
N. sanderae Hort.	4 (L, V–DifCSp; S, V–CVeE–CM)	4 (L, V–BlNSpPe; S, V–N or L, V–CSp; S, V–CM)
N. solanifolia Walp.	4 (L, V–O; S, V–CSp)	4 (L, V–O; S, V–CM)
N. sylvestris Spegaz. and Comes	4 (L, V–O; S, V–CM)	4 (L, V–O; S, V–CM)
N. tabacum L.	4 (L, V–CSpE; S, V–CM–E)	4 (L, V–CSp; S, V–CVePu–CM–Dis)

TABLE 1.—(*Concluded*)

Family and species	Results of inoculation[a, b]	
	With tobacco-etch virus	With tobacco-mosaic virus
N. tomentosa Ruiz and Pav.	4 (L, V–O; S, V–CSp)	4 (L, V–CSp; S, V–CSp–CM–Dis–En)
N. tomentosiformis Goodspeed	0	4 (L, V–CSp; S, V–CM–CSp–En)
N. trigonophylla Dun.	4 (L, V–O; S, V–CM)	4 (L, V–CSp–Ch; S, V–CVe–C–CM–Dis–Stu)
N. undulata Ruiz and Pavon	4 (L, V–C–LeCol; S, V–Stu–C–CM–LeCol–D)	4 (L, V–NSp; S, V–N or L, V–C; S, V–CVe–CM–Dis–Stu)
N. wigandioides Koch and Fint	0	1
Nierembergia hippomanica Miers	1	4 (L, V–O; S, V–CM–Dis)
Petunia hybrida Vilm.	4 (L, V–O; S, V–CM–Dis)	4 (L, V–O; S, V–C–CM)
Physalis alkekengi L.	1	4 (L, V–O; S, V–CM) or 2
P. angulata L.	4 (L, V–NSp; S, V–CM–Dis)	4 (L, V–WNRi–LeAb; S, V–N–LeAb)
P. peruviana L.	4 (L, V–BrNSpPe; S, V–NSp–Wi–LeAb–CM or D)	4 (L, V–O; S, V–CM)
P. subglabrata Mackenzie and Bush	2	2
Salpiglossis sinuata Ruiz and Pavon	4 (L, V–CSp; S, V–ExStu–LePu–CM)	4 (L, V–C–N; S, V–C–NStStr–D)
Schizanthus pinnatus Ruiz and Pavon	4 (L, V–O; S, V–C–N–YoLeCol)	4 (L, V–NSp–PeStStr; S, V–Stu–Wi–N–NSt–D)
Solanum capsicastrum Link	1	3 (L, V–BrNSp; S, Va–O)
S. dulcamara L.	0	1
S. integrifolium Poir	1	4 (L, V–BrNSp–StN; S, V–BrNSp–PeNStr)
S. melongena L.	1	4 (L, V–CSp; S, V–CM) or 4 (L, V–BrNSp; S, V–N–StPeMvNStr–D) or 3 (L, V–BrNSp; S, Va–O)
S. nigrum L.	4 (L, V–O; S, V–CM)	4 (L, V–O; S, V–GCM)
S. sanitwongsei Craib	0	4 (L, V–O; S, V–Ch) or 2
S. tuberosum L.	4 (L, V–O; S, V–CM)	4 (L, V–N; S, V–N)
UMBELLIFERAE		
Apium graveolens L.	0	1
Daucus carota L.	0	1
Pastinaca sativa L.	0	0
Petroselinum hortense Hoffm.	0	1
Trachymene caerulea R. Grah.	0	0
VERBENACEAE		
Lantana camara L.	0	0
Verbena canadensis Britt.	0	1
V. hybrida Voss.	2	3 (L, V–CSp or NSe; S, Va–O)
V. venosa Gill. and Hook.	0	0

of necrotic primary lesions by one virus in areas already systemically involved in patterns of chlorotic mottling by the other would be expected from our knowledge of other dissimilarities between the two viruses but has not been specifically demonstrated heretofore.

In table 1 and in the text immediately following this paragraph, species names of plants for which complete data with respect to susceptibility to infection by both tobacco-etch and tobacco-mosaic viruses became available during the course of the study have been arranged alphabetically by families. Insusceptibility to either virus is indicated in the appropriate column of the table by the symbol 0, susceptibility by numbers 1 to 4, indicating different grades of disease as explained in a footnote to the table; details of recovery of virus and of manifestations of disease at the site of inoculation and in the growing tops of inoculated plants are described parenthetically where needed, by a set of symbols patterned on those of Grant (5) and Wellman (18). These symbols in parentheses, explained in a second footnote of the table, are used primarily for brevity but permit also an adequacy of detail that should facilitate selection of appropriate hosts for specified reactions desired in experimental work. Reisolation after 10 days of any etch virus or of enough tobacco-mosaic virus to produce at least 10 lesions on an inoculated plant of *Nicotiana glutinosa* forms the basis for indication of virus recovery in the table. Experience has shown that such criteria are, in general, appropriate for distinguishing potentially susceptible from insusceptible species. It is to be understood, of course, that a species appearing insusceptible might be infected eventually if attempts to inoculate it mechanically were continued or if grafting or other additional means of inoculation were used, but it is not known that this actually would occur. Obviously, too, a species found potentially susceptible, as shown by experimental infection after mechanical inoculation, may or may not ordinarily become infected in nature.

Families in which all tested species were found insusceptible to infection by both tobacco-etch and tobacco-mosaic viruses were the following (the tested species are also indicated): ACANTHACEAE—*Thunbergia alata* Bojer; AMARYLLIDACEAE—*Hippeastrum puniceum* Urban; APOCYNACEAE—*Vinca rosea* L.; ARACEAE—*Symplocarpus foetidus* (L.) Nutt.; ASCLEPIADACEAE—*Asclepias curassavica* L., *A. syriaca* L.; BEGONIACEAE—*Begonia semperflorens* Link and Otto; CUCURBITACEAE—*Citrullus vulgaris* Schrad., *Cucumis sativus* L., *C. melo* L., *Cucurbita maxima* Duchesne, *C. pepo* L., *Luffa acutangula* Roxb., *Momordica charantia* L.; DIPSACEAE—*Scabiosa atropurpurea* L., *S. caucasica* Bieb.; EUPHORBIACEAE—*Acalypha virginica* L., *Euphorbia heterophylla* L., *E. marginata* Pursh, *E. preslii* Guss., *Ricinus communis* L.; GRAMINEAE—*Avena sativa* L., *Holcus sudanensis* (Stapf) Bailey, *Hordeum vulgare* L., *Secale cereale* L., *Triticum aestivum* L., *Zea mays* L.; HYPERICACEAE—*Hypericum boreale* (Britton) Bicknell; LILIACEAE—*Allium cepa* L., *Lilium philippinense* Baker; LINACEAE—*Linum flavum* L.,

L. grandiflorum Desf., *L. perenne* L.; MALVACEAE—*Althaea rosea* Cav., *Gossypium hirsutum* L., *Hibiscus esculentus* L., *H. manihot* L., *Lavatera trimestris* L., *Malope trifida* Cav., *Malva rotundifolia* L.; NYCTAGINACEAE—*Mirabilis jalapa* L.; ONAGRACEAE—*Clarkia elegans* Dougl., *Oenothera lamarckiana* Ser.; OXALIDACEAE—*Oxalis corniculata* L., *O. stricta* L.; RANUNCULACEAE—*Aquilegia caerulea* James, *Delphinium cultorum* Voss, *Ranunculus asiaticus* L.; RESEDACEAE—*Reseda odorata* L.; ROSACEAE—*Geum canadense* Jacq., *G. chiloense* Balb., *Potentilla arguta* Pursh, *P. monspeliensis* L., *Rosa odorata* Sweet; VIOLACEAE—*Viola arvensis* Murr.

Families in which all tested species were found insusceptible to tobacco-etch virus and in which all tested species showed increase of tobacco-mosaic virus locally but neither systemic spread of virus nor production of an obvious disease were the following (the tested species are also indicated): BIGNONIACEAE—*Incarvillea variabilis* Batalin; BORAGINACEAE—*Anchusa azurea* Mill., *A. capensis* Thunb., *Cynoglossum amabile* Stapf and Drummond, *Echium vulgare* L., *Heliotropium corymbosum* Ruiz and Pav., *Myosotis scorpioides* L., *M. sylvatica* Hoffm.; CRASSULACEAE—*Kalanchoë daigremontiana* Hamet and Perrier; FUMARIACEAE—*Adlumia fungosa* Greene; PLUMBAGINACEAE—*Limonium bonduelli* (Lest.) Kuntze, *L. sinuatum* (L.) Mill.; POLYGONACEAE—*Fagopyrum esculentum* Gaertn., *Polygonum hydropiper* L., *Rumex crispus* L., *R. obtusifolius* L.; TROPAEOLACEAE—*Tropaeolum majus* L.

It will be observed, by references to the digits in the body of table 1, that in no instance did a plant show evidence of infection and virus multiplication (numbers 1 to 4) by reason of mechanical inoculation with tobacco-etch virus and failure (indicated by 0) to become infected when similarly inoculated with tobacco-mosaic virus. However, the grades of disease were not so clearly correlated, one virus sometimes outrunning the other in moving throughout the plant (as shown by combinations of grade-of-disease numbers such as 1–4, 4–1) or outdoing the other in injury to invaded tissues (1–3, 3–1). Whatever one's estimate of the practical importance of masked carriers as virus reservoirs, a further importance attaches to them from a theoretical viewpoint. Presumably masked carriers of a virus, equally with obviously diseased plants, reflect at least the minimal biological requirements for increase of the virus within its host. It is true that severity of disease has been considered by Leach (13) to influence insect vectors in their transmission of viruses from diseased to healthy plants. Thus the perpetuation of viruses in nature may be dependent in some measure on degree of severity of disease manifestations. Yet plants obviously affected by disease after infection together with those in which virus multiplies but in which effects on the plant are so slight as to be overlooked on casual examination must be collectively recognized as defining, for theoretical purposes, the distribution in nature of materials and conditions essential for viral increase.

Reference to figure 2 will show that the experimental host range of etch

virus is centered about a part of the plant kingdom near the Solanaceae, much as is that of the mosaic virus; further, that it is narrower than, and enclosed within, that of the mosaic virus as has been noted in discussing table 1. Although all known naturally infected hosts of etch virus are members of the Solanaceae, it must be noted that the Hydrophyllaceae as a group are notable for susceptibility so far as tested experimentally. Widespread cultivation of solanaceous plants perhaps has been the decisive factor in drawing our attention to etch disease, but the causative virus long may have utilized wild species in the Solanaceae and in other families as hosts, and infections in nature may yet be found in hydrophyllaceous species or in closely related groups.

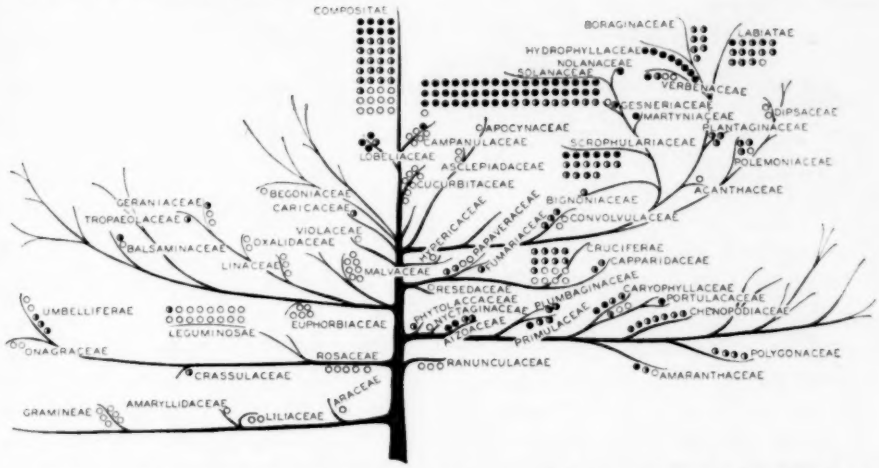

FIG. 2. Taxonomic relationships of plants susceptible to infection by tobacco-etch and tobacco-mosaic viruses. Species tested by inoculation are indicated by circles; black circles indicate species found susceptible to both viruses; white circles, to neither virus; half black circles, to tobacco-mosaic, but not to tobacco-etch virus; no species was found susceptible to tobacco-etch virus yet not to tobacco-mosaic virus.

Figure 2 emphasizes the fact that the experimental host ranges of tobacco-etch virus and of tobacco-mosaic virus are on the whole familial in character and tend to involve large blocks of species in closely related families, exceptional instances of resistance within these blocks being few, exceptional instances of susceptibility in other families being likewise few. Arrangement of families in the diagram is like that in diagrams used in an earlier paper (7) dealing with experimental hosts of tobacco-mosaic virus alone.

In table 2 there is a brief summary of the incidence of various types of response among the tested species. From this tabulation may be derived three generalizations other than the principal one that species incapable of supporting the increase of tobacco-mosaic virus proved incapable also of supporting the increase of tobacco-etch virus. These are: Species that showed systemic symptoms (Class 4) with mosaic virus mostly showed systemic symptoms with etch virus also; the reason for this is not evident, but the converse was true as well, for species that showed systemic symptoms

with etch virus mostly showed systemic symptoms with the mosaic virus as well. Species that did not take etch but did take mosaic tended to take mosaic mildly; why should this have been? Finally, species that took mosaic mildly mostly did not take etch.

Inspection of the classes of response (0 to 4) listed in table 1 and accompanying text suggests that for both viruses natural immunity must be considered the commonest of the five arbitrarily defined conditions of herbaceous plants in general. For tobacco-mosaic virus, the other four types of response in order of frequency were 1, 4, 3, 2, the largest number of susceptible plants localizing virus without obvious disease manifestations. For tobacco-etch virus, however, the order of frequency was 4, 1, 2, 3, most susceptible plants permitting systemic spread of virus and clearly showing disease. If the production of obvious systemic disease had been regarded

TABLE 2.—*Number of species affected by different grades of disease after testing separately with tobacco-etch and tobacco-mosaic inoculations*

Grades of disease[a]	0 (*etch*)	1 (*etch*)	2 (*etch*)	3 (*etch*)	4 (*etch*)
0 (*mosaic*)	111	0	0	0	0
1 (*mosaic*)	80	7	3	3	7
2 (*mosaic*)	5	1	4	0	5
3 (*mosaic*)	20	2	1	2	2
4 (*mosaic*)	11	5	0	2	39[b]

[a] Symbols 0 through 4 for arbitrary grades of disease as in table 1.

[b] In a few instances plants of a given species have reacted at times with one grade of disease, but at other times more severely; the more severe grade is entered in this table for each such case.

as the only criterion of infection, it would have been concluded that the two viruses affected about equal numbers of the species inoculated (53 for severe-etch virus; 57 for tobacco-mosaic virus). Actually, however, this is far from the case when the more tolerant and the movement-restraining hosts are also recognized, 73 per cent of tested species proving naturally immune on inoculation with etch virus, only 36 per cent on inoculation with tobacco-mosaic virus.

Altogether, among the 310 species for which comparative data have been obtained, 83 proved susceptible to infection by both viruses, 116 to infection by tobacco-mosaic and not tobacco-etch virus, none to infection by tobacco-etch and not tobacco-mosaic virus, 111 to infection by neither virus.

DISCUSSION

On the basis of the present results, it seems feasible to predict that species susceptible to infection by strains of etch virus will usually, perhaps always, prove susceptible to infection by strains of tobacco-mosaic virus.

Such predictability characterizes no other pair of viruses that are serologically unrelated, confer no cross immunity, and have radically different resistances to inactivation by heat.

What is the meaning of this finding that all 83 species that proved susceptible to infection by etch virus were able to support increase of tobacco-mosaic virus? Are the requirements of these two viruses definitely related as has been suggested with respect to the requirements of some viruses (1, p. 117)? How should we explain the ability of tobacco-mosaic virus to infect an additional 116 species that appear to be naturally immune to infection with etch virus?

The experimental evidence now in hand suggests the hypothesis that the tested (severe-etch) strain of tobacco-etch virus requires one or more substances or conditions in its hosts in addition to all those needed by the strain used to represent tobacco-mosaic virus. In the course of time this hypothesis will be tested automatically, as information on additional species accumulates. It may be worthwhile to remark that exceptions naturally must be expected to arise in connection with any such generalization. If they prove few, however, they may tend merely to emphasize the significance of the rule without disproving it, for they may point only to independent relationships, such as specific harmful effects on tobacco-mosaic virus even in the presence of all that is normally required for its increase.

A greater similarity than might have been anticipated was found in the types of disease induced by tobacco-etch and tobacco-mosaic viruses in the experimental hosts as a group. The most distinctive characteristic of etch disease in tobacco, a whitish stippling of areas of leaf surface involved in primary and secondary lesions, has given rise both to the name of the causative virus (17, 12) and to a tendency to consider the virus as generically distinct from tobacco-mosaic virus (16). Reference to table 1, however, will show that chlorotic mottling (CM) was commonly observed in this study in systemically susceptible hosts, etching (E) very rarely. Etch virus appears, on the whole, to be a typical mosaic-type virus, rarely deviating even to the extent of producing etch patterns. Indeed the diseases induced by the two viruses seem fundamentally alike, and are often strikingly similar in their manifestations.

With this similarity of disease type and the partial predictability of host range in mind, one might tend to conclude that the two viruses were closely related genetically. Evidence against any very close relationship, however, may be summarized as follows: No interference with production of tobacco-mosaic virus was noted by Bawden and Kassanis in tests involving previous inoculation of plants with severe-etch virus (1, p. 113). No protection against local necrosis is afforded by inoculation of appropriate plants with one before the other (Fig. 1). There is no known antigenic similarity (3) despite an early erroneous report (2). Moreover, the two viruses differ sharply in their thermal stabilities; whereas tobacco-mosaic virus must be held at about 92° C. for 10 minutes to be completely inactivated, severe-etch

virus is inactivated at about 53° to 55° C. in the same length of time (1, p. 115; 10). Such numerous and fundamental differences would suggest a distant rather than a close relationship, if one exists.

In view of the evidence above detailed and because no other two viruses of wide host range are known to have so predictable a relationship between their hosts, it may be most feasible for the present to consider these two viruses with their respective strains and substrains as fairly closely allied, but distinct, members of the group of typical mosaic-disease viruses. This is already implied by their inclusion in a common genus as separate species (8).

SUMMARY

Among 310 plant species tested by inoculation with tobacco-etch and tobacco-mosaic viruses, 83 proved capable of supporting increase of both viruses, 111 proved incapable of acting as hosts of either virus, 116 were susceptible to attack by the mosaic though not by the etch virus, but none were suceptible to infection by the etch that were not susceptible to infection by the mosaic virus.

Types of disease induced in susceptible species are briefly recorded.

It is suggested that inclusion of the whole known potential host range of tobacco-etch virus within that of tobacco-mosaic virus may mean that the requirements of the former include all the requirements of the latter as well as some other requirement or requirements.

The earlier finding that plants susceptible to infection by tobacco-mosaic virus are largely confined to closely related families is confirmed. A similar but somewhat narrower host range is indicated for tobacco-etch virus.

Tobacco-etch and tobacco-mosaic viruses, though producing similar mottling diseases in many plants, including tobacco, are believed not to be closely related because they are immunologically and serologically distinct and definitely unlike in thermal stability. No other two viruses of so wide host range are known, however, to have so predictable a relation with respect to what plants they can infect. It is suggested that the similarity in their host requirements (which by no means amounts to an identity even in this characteristic), taken in conjunction with the general similarity of their induced diseases, may be considered as evidence of a relationship of a somewhat more distant type than that displayed by varieties of a single virus.

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HELMINTHOSPORIUM TURCICUM LEAF BLIGHT OF CORN

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(Accepted for publication April 29, 1946)

The 1942 epidemic of *Helminthosporium turcicum* leaf blight came at a time when hybrid corn was largely replacing the old open-pollinated varieties. Many new lines and crosses were being developed and tested under different environmental conditions. Some were severely injured by leaf blight while other lines and crosses appeared to be more resistant. The double crosses in use in the Corn Belt were relatively susceptible. The differences observed indicated the advisability of further tests for resistance and of selecting resistant lines for use in the corn breeding program.

In 1944, as a result of field inoculations at Beltsville, Maryland, infection was produced that was even heavier than that in 1942. Some of the same lines and crosses were grown in both years. The results of the 1944 inoculations are given here in comparison with the results of natural infection in 1942.

METHODS OF INOCULATION

The 1944 field inoculations at Beltsville were made on 200 inbred lines, 176 single crosses, and 184 double crosses, assembled from the corn-growing areas, to test their relative susceptibility to leaf blight. These lines and crosses were grown in single-row plots of approximately 24 plants each in 3 or 4 replications, altogether covering about 5 acres.

Five out of 70 isolations of *Helminthosporium turcicum* Pass., from naturally infected inbred lines of dent corn grown in 1942 at Beltsville, were selected for rapid growth of mycelium, abundant spore production, and virulence. Single-spore cultures of all 5 were combined for the inoculations.

The cultures first were grown on oat hulls plus a small amount of corn meal in Petri dishes; and oat hulls transferred to potato-dextrose agar plates for spore production. An abundance of spores developed in about 3 weeks. Spore suspensions were made by running ten, 100-mm. Petri-dish cultures in one liter of water through an homogenizer[1] for 2 minutes. This suspension was stored at 5° C. until needed. The suspension was diluted 1 to 10 in the field and about 10 ml. sprayed into the central coil of leaves of each plant. Inoculations were begun June 23 when the plants were 12 to 18 inches tall and repeated about twice weekly until the plants began to tassel about the middle of July.

RESULTS OF THE INOCULATIONS

The final development of leaf blight, following inoculations in 1944, was even heavier than the epidemic of natural infection in 1942. Within a week after each inoculation, numbers of small water-soaked, yellow dots, 1 to 2 mm. in diameter, appeared in bands 2 to 3 inches wide, on the de-

[1] Manufactured by Eppenback, Inc., Long Island City, New York, Serial No. 3923.

veloping leaves. On some lines of corn these developed into typical *Helminthosporium turcicum* lesions. On other lines no further development took place. Until the last week in July little, if any, spreading of the disease from these original infections could be observed. For nearly a month following the first inoculation on June 23, there was almost no rain. Rains of 0.46 inches and 1.08 inches fell July 13 and July 20, respectively. On August 3, more than 5 inches of rain fell. Heavy dews formed at night. Leaf blight began to spread rapidly and continued to spread over all corn on the Station farm in the vicinity of the inoculation plots during the remainder of the growing season.

It had been hoped that the leaf blight would develop sufficiently from the initial inoculations so that plants, in segregating progenies, could be

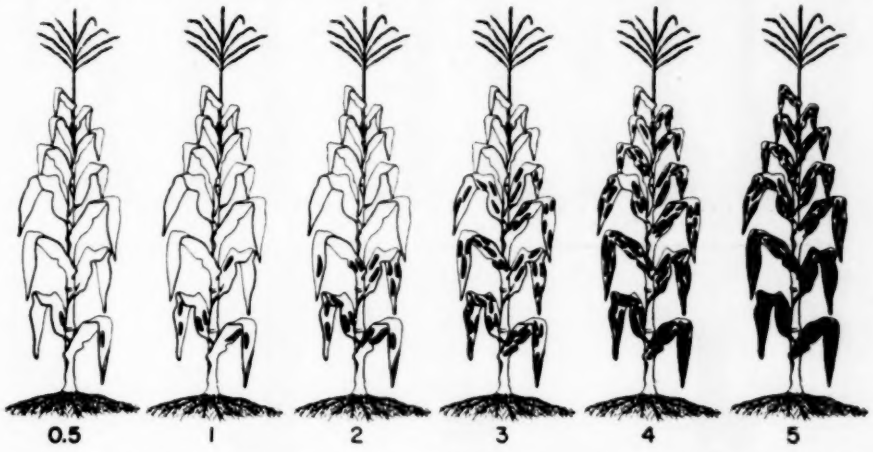

FIG. 1. Scale for rating *Helminthosporium turcicum* leaf blight. 0.5, Very slight infection, one or two restricted lesions on lower leaves; 1, Slight infection, a few scattered lesions on lower leaves; 2, Light infection, moderate number of lesions on lower leaves; 3, Moderate infection, abundant lesions on lower leaves and few on middle leaves; 4, Heavy infection, lesions abundant on lower and middle leaves and extending to upper leaves; 5, Very heavy infection, lesions abundant on all leaves, plants may be prematurely killed.

classified as to susceptibility by the time they flowered, and thus lighten the task of hand pollination in the program of breeding for resistance. Differences among strains of corn and among plants in the amounts of infection that developed directly from inoculation, however, were entirely unsatisfactory for this purpose. Fortunately the disease started to spread during tasseling and the infection from this secondary spread was sufficiently heavy at pollinating time to permit a reasonably accurate selection of resistant plants for breeding operations.

The amount of blight infection was recorded in 6 classes (Fig. 1).[2] Plants with only a trace of infection were rated 0.5 and those with pro-

[2] Ullstrup, A. J., P. E. Hoppe, and Charlotte Elliott. Report of the committee on methods for reporting corn disease ratings. U. S. Dept. Agr., Agr. Res. Admin., Bur. Plant Indus., Soils, and Agr. Engin., Div. Cereal Crops and Dis. 23CC, 5 pp., Feb., 1945. [Processed.]

gressively heavier infection were rated 1, 2, 3, 4, or 5, the 5 representing very heavy infection. Ratings were made on a plot basis the latter part of August and the middle of September.

The ratings on 95 inbred lines, grown both in 1942 and 1944, are shown in table 1. In 1944 the inbred lines were divided into two groups, those of Corn-Belt maturity (about 125 to 145 days) being grown in one series and the later-maturing lines in another. The ratings show much less infection

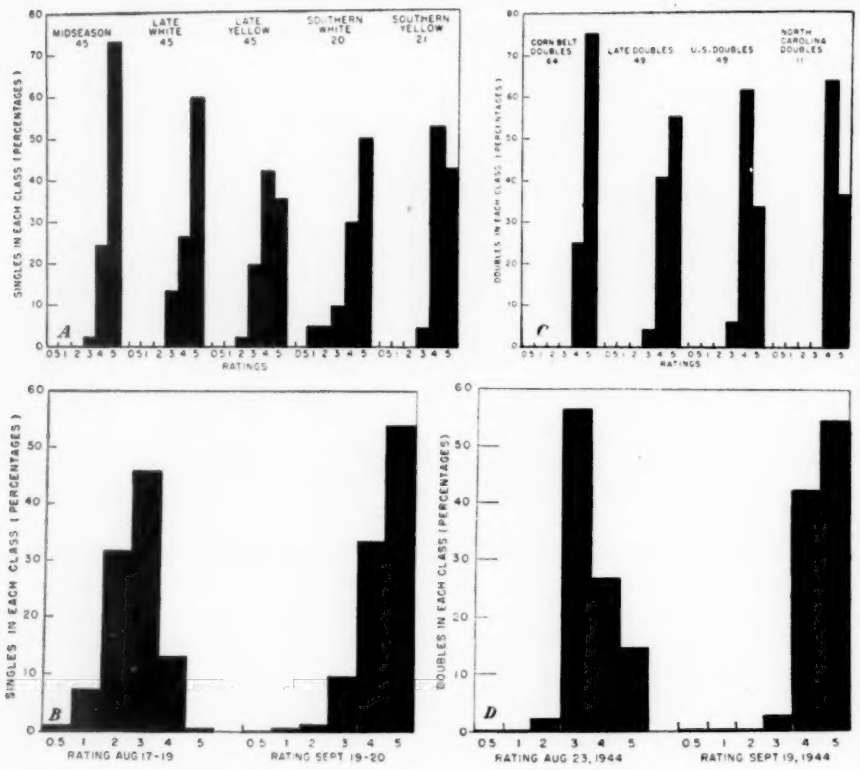

FIG. 2. Frequency distributions of ratings of *Helminthosporium turcicum* leaf blight in single and double crosses of dent corn at Beltsville, Md., 1944. A, 176 single crosses rated September 19–20; B, same single crosses as in A, rated August 17–19 and September 19–20; C, 173 double crosses rated September 19; D, same double crosses as in C, rated August 23 and September 19.

in 1942 than in 1944, but on the whole there is reasonable agreement between the results obtained in the two seasons, certain lines having low ratings in both years and others high. There is, however, no perfect correlation. K64 and Tx155A have just as high ratings in 1942 as in 1944, whereas Ky27, K60, Oh56 with relatively low ratings in 1942 had very high ratings in 1944. Whether these discrepancies are due to differences between strains of the lines used in the 2 years or are due to differences in races of the fungus or to other causes remains to be determined. Only 3 of the lines of Corn-Belt maturity tested, and only 8 of the late inbred lines had light infection

TABLE 1.—*Helminthosporium turcicum leaf blight ratings on inbred lines of corn in 1942 and 1944 at Beltsville, Maryland*

Lines of Corn-Belt maturity	Ratings			Late inbred lines	Ratings		
	1942	1944			1942	1944	
	Aug. 12	Aug. 25	Sept. 16		Aug. 12	Aug. 24	Sept. 15
Tx206	0.5	2.5	2.8	NC34	0.5	0.5	0.6
Tx155A	3.0	3.0	3.0	CI.23	0.5	0.9	1.1
Tx4R3	0.5	3.0	3.8	K175	0.5	0.9	1.5
Tx203	3.0	3.0	3.8	T49B	0.5	1.5	2.3
KB397	1.0	3.0	4.0	T105B	0.5	1.8	2.5
90	3.0	3.8	4.0	K155	0.5	2.0	2.5
Tx61M	1.0	4.0	4.0	T61	0.5	1.8	3.0
L317	0.5	3.3	4.3	T7–6A	0.5	2.0	3.0
B2	1.0	4.0	4.5	T18	1.0	2.8	3.3
CI.4–8	1.0	4.0	5.0	K54	1.0	3.0	3.3
K	1.0	4.0	5.0	Ky122	0.5	2.0	3.5
WF9	2.0	4.0	5.0	K41	0.5	3.0	3.8
Bl 345	2.0	4.0	5.0	Ky58	0.5	3.0	3.8
Pr	2.0	4.0	5.0	T13	0.5	2.0	4.0
Oh56	0.5	4.0	5.0	Kys	1.0	2.8	4.0
461–3	0.5	4.3	5.0	CI.43	0.5	3.0	4.0
38–11	3.0	4.8	5.0	K64	4.0	3.0	4.0
33–16	1.0	4.8	5.0	K148	0.5	3.0	4.0
Oh07	2.0	4.8	5.0	K158	1.0	3.0	4.0
Oh51	1.0	4.8	5.0	K4	1.0	3.3	4.0
CI.187–2	2.0	5.0	5.0	CI.5	1.0	3.5	4.0
CI.540	2.0	5.0	5.0	K166	2.0	4.0	4.0
A	3.0	5.0	5.0	CI.24	1.0	3.0	4.3
Hy	2.0	5.0	5.0	CI.63	0.5	3.5	4.3
M14	2.0	5.0	5.0	T85	0.5	4.0	4.3
R4	3.0	5.0	5.0	CI.21	1.0	4.0	4.5
5120	3.0	5.0	5.0	Ky39	0.5	3.3	4.8
P8	3.0	5.0	5.0	CI.41	3.0	4.0	4.8
Tr	4.0	5.0	5.0	Ky27	0.5	4.0	4.8
66	2.0	5.0	5.0	MoG	1.0	4.0	4.8
Bl 349	2.0	5.0	5.0	CI.2	1.0	3.5	5.0
Mc401	4.0	5.0	5.0	Ky17	1.0	3.8	5.0
Os420	3.0	5.0	5.0	CI.27	1.0	4.0	5.0
ITE701	5.0	5.0	5.0	CI.7	2.0	4.0	5.0
Oh01	3.0	5.0	5.0	K10	3.0	4.0	5.0
Oh67	3.0	5.0	5.0	K180	1.0	4.0	5.0
Oh84	2.0	5.0	5.0	Ky41	3.0	4.0	5.0
Tx127C	4.0	5.0	5.0	Mo940	0.5	4.0	5.0
				T7–2E	0.5	4.0	5.0
				K18	1.5	4.3	5.0
				Ky49	2.0	4.3	5.0
				Mo7Ra	1.0	4.3	5.0
				CI.1	1.0	4.5	5.0
				K124	3.0	4.5	5.0
				Ky13	1.0	4.5	5.0
				Ky21	2.0	4.5	5.0
				T21A	2.0	4.5	5.0
				K17	1.0	4.8	5.0
				K159	3.0	4.8	5.0
				Ky56	1.0	4.8	5.0
				CI.3	3.0	5.0	5.0
				CI.6	4.0	5.0	5.0
				K60	2.0	5.0	5.0
				K124	3.0	5.0	5.0
				K151	2.0	5.0	5.0
				Ky132	3.0	5.0	5.0
				T14	3.0	5.0	5.0

or less in 1944. Of these, one of the lines of Corn-Belt maturity and six of the late lines are listed in table 1. The other four, namely, CI.15, CI.19, Ky114, and Mo21A, were not tested in 1942. All other lines had moderate to very heavy infection. There was less infection in the late lines in both years than in the Corn-Belt lines.

Five groups of single crosses, designated as midseason, late white, late yellow, southern white, and southern yellow crosses, were included in the 1944 experiments. The first three groups each contained all of the 45 possible combinations among 10 parent lines and the last two each contained all but one and all, respectively, of the 21 possible combinations among seven parent lines. Frequency distributions of the ratings for the single crosses in each of the different groups are shown in figure 2, A. In September the midseason single crosses had more very heavy infection than the late and the southern single crosses. The southern white singles had 5 per cent very light and 5 per cent light infection. The late yellow singles are the only others with any light infection. Frequency distributions of the ratings for all 176 singles in August and in September are shown in figure 2, B. Eighty-seven per cent of all single crosses tested in 1944 were heavily infected in September.

The 1944 leaf blight ratings of inbred parents of each group of single crosses and the mean ratings of the single crosses involving each parent line are reported in table 2. K155 was the most resistant of any of the parents of the midseason group and the mean August and September ratings of the single crosses involving this line also were lower than those for any other parent line. The low September rating of Mo22 and the low September average rating for the crosses involving this line indicate that it is probably the most resistant line in the late white group. In the late yellow group K155 again was the most resistant parent line both when judged on its ratings as an inbred line and on the average ratings of the single crosses involving it.

In the southern white group NC34 was the most resistant parent line and the crosses involving it had the lowest average ratings. NC34 had the lowest rating of all the 200 inbred lines. It was significantly more resistant than any other line tested. In the southern yellow group there were no marked differences.

An attempt was made in 1944 to test as many double crosses as were available. The double crosses tested were divided into four groups and these were designated as Corn-Belt doubles, late doubles, U. S. doubles, and North Carolina doubles, respectively. Frequency distributions of the ratings of these double crosses are given in figure 2, C. There was no light infection in any of the double crosses in the September rating, and only 4 per cent moderate infection in the late doubles and 6 per cent in the U. S. doubles in September. The Corn-Belt doubles and the North Carolina doubles were 100 per cent heavily infected, the late doubles and the U. S. doubles were 96 per cent and 94 per cent heavily infected, respectively.

TABLE 2.—*Summary of leaf blight ratings of inbred lines and the mean ratings of their single crosses following inoculation with Helminthosporium turcicum in 1944 at Beltsville, Maryland*

Inbred lines	Ratings Aug. 17–19		Ratings Sept. 19–20	
	Means of crosses[a]	Parent line[b]	Means of crosses[a]	Parent line[b]
Midseason group				
Hy	3.1	5.0	4.7	5.0
L317	2.6	3.3	4.7	4.3
WF9	3.1	4.0	4.9	5.0
38–11	3.1	4.8	4.7	5.0
B3	3.1	.....	5.0	.....
461–3	2.9	4.3	4.9	5.0
K155	1.8	2.0	4.0	2.5
N6	2.3	.....	4.7	.....
Oh28	3.0	.....	4.6	.....
CI.7	3.3	4.2	4.9	5.0
Late white group				
33–16	3.0	4.8	4.4	5.0
Ky27	2.1	4.0	4.1	4.8
K55	2.8	.....	4.9	.....
K64	2.7	3.0	4.4	4.0
H25	2.6	4.3	4.6	5.0
H24	2.7	4.5	4.7	4.8
R30	3.1	.....	4.8	.....
Mo22	1.8	3.0	3.4	3.0
Tx4R3	1.9	3.0	4.3	3.8
T18	1.6	2.8	4.2	3.3
Late yellow group				
38–11	3.0	4.8	4.3	5.0
H7	2.4	.....	4.5	.....
K223A	3.2	.....	4.8	.....
K4	2.3	3.3	4.0	4.0
Kys	2.4	2.8	4.1	4.0
K155	1.8	2.0	3.3	2.5
Oh04	2.9	4.0	4.1	5.0
T8	2.1	3.0	3.4	3.5
CI.5	2.3	3.5	3.9	4.0
CI.7	3.2	4.0	4.6	5.0
Southern white group				
L13	3.2	.....	4.3	.....
NC34	1.3	0.5	3.1	0.6
NC37	2.7	4.0	4.6	5.0
R7	3.9	.....	4.9	.....
T10	2.5	4.5	4.3	5.0
T61	2.1	1.8	3.8	3.0
Tx155A	2.9	3.0	4.2	3.0
Southern yellow group				
R34	2.7	.....	4.5	.....
R40	3.2	4.0	4.5	4.0
Kys	2.5	2.8	4.0	4.0
K4	2.7	3.3	4.0	4.0
NC12	2.5	3.0	4.2	4.0
CI.7	3.2	4.0	4.9	5.0
CI.21	3.2	4.0	4.5	4.8

[a] Each rating in these columns is the average of the ratings on the single crosses produced by crossing each inbred line listed on the left with all of the other lines of the same group.

[b] Ratings on the parental inbred lines themselves.

Frequency distributions of the August and September ratings of 173 double crosses are given in figure 2, D. There was 2.3 per cent light infection in August but none in September; 56 per cent had moderate infection in August with only 2.9 per cent in September; 41 per cent had heavy infection in August, and 97 per cent were heavily infected in September.

The results of these inoculations indicate that there are resistant lines that tend to transmit their resistance to crosses, that earlier lines and crosses are more susceptible than later lines and crosses, and that the double crosses now being used in hybrid corn production are susceptible to leaf blight. NC34 was outstanding in its resistance to *Helminthosporium turcicum* leaf blight and in transmitting this resistance to its hybrids. It is possible that differences in genetic composition of the host, differences in virulence of the fungus, or other factors may complicate the problem of resistance to this disease. Abundance of moisture appears to determine the stage in the development of the plant when the leaf blight becomes severe and the resulting damage to the crop. If the disease develops early in the season when corn plants are only partly grown, the entire crop may be destroyed. If heavy infection develops late in the season, after the ears are well formed and the plants are approaching maturity, losses may be restricted largely to the fodder or may affect both the yields and quality of fodder and of grain.

SUMMARY

Field inoculation experiments were conducted at Beltsville, Md., in 1944 to determine relative susceptibility of inbred lines and crosses of dent corn to *Helminthosporium turcicum* leaf blight. Results of these inoculations were compared with records of leaf blight during the epidemic of 1942.

About 5 acres of corn were inoculated twice weekly beginning the last of June and ending as the plants came into tassel. The disease spread rapidly during August and September.

Leaf blight ratings on 200 inbred lines, 176 single crosses, and 184 double crosses indicate that most lines and crosses are susceptible. NC34 was significantly more resistant than any other line tested. CI.23, K175, Ky114, Mo21A, T49B, T105B, K155, CI.15, CI.16, and Tx206 had only traces to light infection. The other lines tested showed moderate to very heavy infection. In general, the resistance of resistant lines was transmitted to their hybrids.

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A METHOD FOR THE MACROSCOPIC STUDY OF ROOT DISEASES

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(Accepted for publication April 30, 1946)

Pirone[2] described a method suitable for the commercial propagation of plants from cuttings without use of soil or of any type of solid medium. A modification of this method seemed to offer possibilities in plant pathology, particularly in the study of root diseases, enabling all parts of the plant to be examined macroscopically at any time during the period of infection. The method has unlimited possibilities in permitting the use of different types of culture media to determine parasitic relationships.

METHOD

Seeds of cotton and corn were germinated under sterile conditions on moist filter paper, and when the hypocotyls had emerged to a length of 2 to 3 centimeters, they were placed in root containers similar to those described by Pirone (Fig. 1). Each container consisted of a metal frame

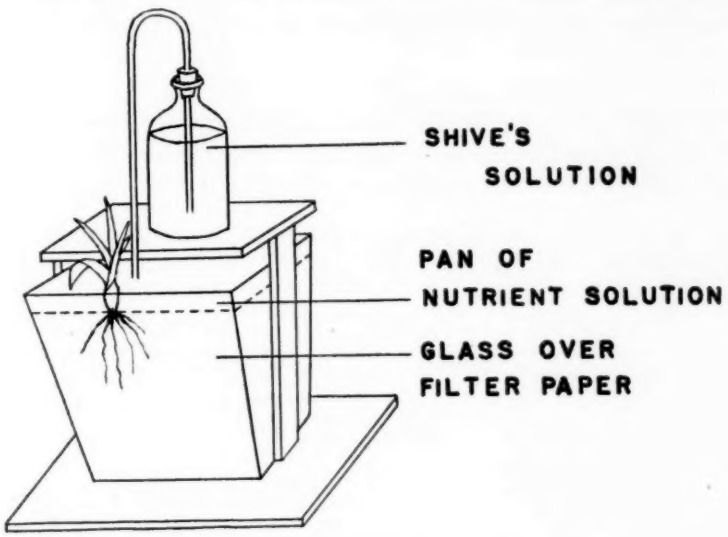

FIGURE 1. ROOT CONTAINER

supporting a glass surface 16 by 24 inches. At the top of the metal frame, supported partially by a wooden frame and partially by the edges of the metal frame, was a metal pan containing nutrient solution. Adjacent to the glass was a strip of filter paper which folded over into the pan containing the mineral solution. The germinating seeds were placed at the top of the

[1] Dr. Goldsmith was Professor of Botany at the University of Texas prior to his death in October, 1943.

[2] Pirone, P. P. A new method of plant propagation. Science 94: 74. 1941.

container between the glass and the filter paper. A black, metal sheet covered the glass portion to prevent light injury to the roots.

The nutrient supplied was Shive's solution[3] consisting of 3 salts, potassium dihydrogen phosphate (0.018 M), calcium nitrate (0.0052 M), and magnesium sulphate (0.015 M).

The cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar, was used as the parasite in all of the experiments. Corn, which is resistant to this fungus in the field, and cotton, which is susceptible, were used as the host plants. The fungus cultures used for inoculation were grown by Dunlap's[4] method in order to insure pathogenicity.

After the plants had grown sufficiently to develop a root system approximately 6 inches long, one series each of cotton and corn was inoculated with fresh sclerotia of the fungus. Another series of each was used as a control.

To determine the possibilities of the root-growing apparatus as a method of testing the effects of controlled nutrient solutions on parasitism, preliminary work was done by substituting Na_2SO_4 for $MgSO_4$ in the Shive's solution, thus creating a magnesium deficiency. Young corn seedlings were placed in the container after 12 hours and were inoculated in the usual manner. They were given the complete Shive's solution for 60 hours and the magnesium-free solution throughout the remainder of the experiment.

RESULTS

The plants were examined daily for symptoms of wilting, and measurements of growth increments of the roots were made whenever practical. Usually this was not possible with cotton roots as they grew too slowly to permit accurate measurements. In general, it was found that corn, which is normally immune from the root-rot fungus in the field, could be attacked but not killed under the conditions of the experiment. Plants which were inoculated were usually retarded in growth and lacked the vigor of the control plants. The measurements are in table 1.

TABLE 1.—*Measurements on corn plants grown in root containers. Five plants were inoculated with Phymatotrichum omnivorum and five were not inoculated*

Measurements[a]	Inoculated plants	Noninoculated plants
Epicotyl: length	23 cm.	30 cm.
green weight	10.3 gm.	29.5 gm.
dry weight	1.3 gm.	3.6 gm.
Hypocotyl: length	27 cm.	43.5 cm.
green weight	1.6 gm.	4.2 gm.
dry weight	.45 gm.	.78 gm.
Number of roots	9	13.5

[a] Each measurement is the average for 5 plants.

[3] Shive, J. W. A three-salt nutrient solution for plants. Amer. Jour. Bot. 2: 157–160. 1915.

[4] Dunlap, A. A. A convenient soil-culture method for obtaining sclerotia of the cotton root rot fungus. Amer. Jour. Bot. 28: 945–947. 1941.

FIGURE 2.
AVERAGE GROWTH OF NORMAL AND INFECTED CORN ROOTS IN SHIVE'S SOLUTION

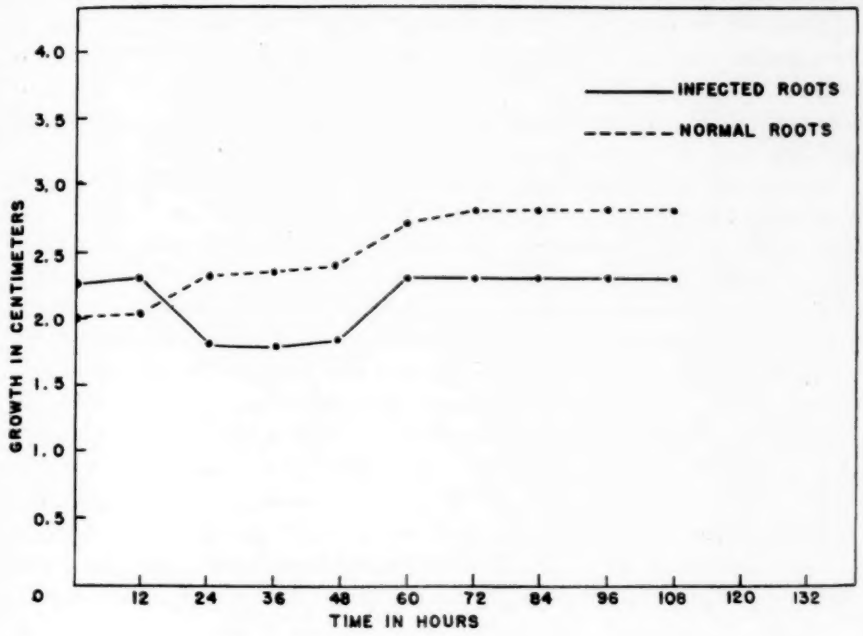

FIGURE 3.
GROWTH OF INFECTED CORN ROOTS IN SHIVE'S SOLUTION

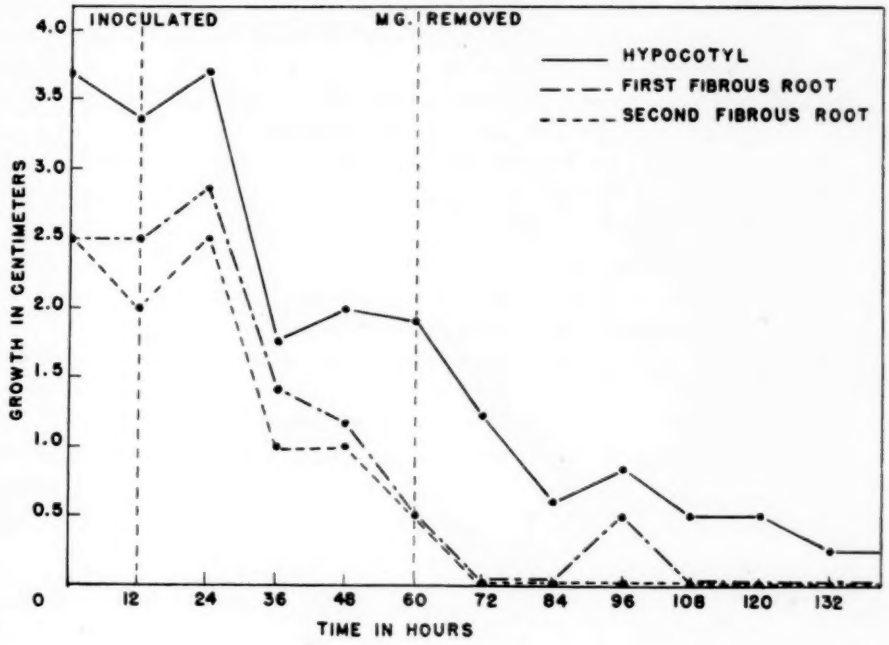

Figure 2 shows the effect of inoculation on the root growth of 5 corn plants as well as the growth of a similar number noninoculated. Measurements are in centimeters and were made at 12-hour intervals. After 36 hours the root-rot fungus caused a constantly diminishing growth rate of the corn roots, although none of the plants was killed during the course of the experiments. In contrast to this, the control plants continued to grow normally.

Cotton seedlings, which are usually immune[5] in the field, gave results similar to those of corn when grown under experimental conditions. The controls continued to grow throughout the experiment while inoculated seedlings showed symptoms of root necrosis very early. The inoculated roots ceased growing 24 hours after inoculation and soon decayed. If there were not other secondary roots to serve as sources of nourishment, the entire plant soon died. Sections through the infected areas showed the root-rot fungus in the cells of the cortex, endodermis, and xylem.

The corn plants which were deprived of magnesium supply 60 hours after inoculation showed even more symptoms than those which were infected in the presence of full nutritional requirements. Growth rates continued diminishing, and after 3½ days root growth ceased. Figure 3 shows the measurements of 3 typical roots during the experiment. Within a week following the cessation of growth, the plants wilted and died.

SUMMARY

1. A macroscopical method for the examination of root infections has been devised.

2. Cotton seedlings growing in root containers were killed after inoculation with *Phymatotrichum omnivorum*.

3. Corn, which is immune from *Phymatotrichum omnivorum* in the field, was rendered susceptible to attack but not killed.

4. Corn seedlings can be rendered susceptible to typical infection by *Phymatotrichum omnivorum*, which results in death, by withholding magnesium from the nutrient solution.

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THE UNIVERSITY OF TEXAS, AUSTIN 12, TEXAS.

[5] Blank, L. M. The susceptibility of cotton seedlings to *Phymatotrichum omnivorum*. Phytopath. 30: 1033–1041. 1940.

GREEN DWARF: A VIRUS DISEASE OF POTATO[1]

J. A. MILBRATH[2]

(Accepted for publication May 17, 1946)

Green dwarf, an undescribed disease of potatoes, has been present in Oregon for a number of years. The disease is caused by a virus which seems to be distinct from all other viruses which have been reported on potatoes. Green dwarf has been collected from Baker, Crook, Deschutes, and Malheur Counties in Oregon. Since it is often found in fields of potatoes being grown for seed, it is probable that the disease is more widely distributed but not recognized. Tuber-perpetuated green dwarf was found in a number of fields of Netted Gem potatoes grown from seed which had been produced in Montana.

SYMPTOMS OF THE DISEASE

Tuber perpetuated. The outstanding symptoms of tuber-perpetuated green dwarf are late emergence and extreme dwarfing. It is not uncommon for a mature green-dwarf potato plant to be only six inches high, while adjacent healthy plants are two feet or more high. The basal leaves appear normal, but are close together in a basal rosette of four or five leaves. The remaining terminal growth is dwarfed and malformed, and the growing point is pinched together in a cluster of small leaves (Fig. 1, A). The leaflets on the young leaves cup upward, forming small boat-like structures (Fig. 1, C). The plants are normal green or sometimes even darker green than normal. The tubers are small, but have no other symptom.

The tuber-perpetuated symptoms of green dwarf in the greenhouse are quite different from those of field-grown plants. Emergence is one to two weeks later than normal and when the sprout finally breaks through the soil a dark green, leafy bud develops, which does not unfold its leaves for some time. The entire plant may not be over one to two inches tall, two or three weeks after emergence (Fig. 2). Gradually the stem elongates and the older leaves expand into small dark green, unmottled structures, often not over one to two inches long. The plants are very stiff and erect, and the growing point remains compact or pinched together. After two months of growth, Green Mountain plants from tubers inoculated the previous year with green dwarf virus, produced blossom clusters on plants not over six inches tall, and the leaf spread of the plants did not exceed two inches (Fig. 1, B). Some plants, especially the White Rose variety, after a late emergence and slow development, will begin to grow normally and produce a plant 12–18 inches tall. These plants appear normal, except that leaves are short and leaflets small, which gives the entire plant a narrow, leggy appearance.

[1] Published as Technical Paper No. 486 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution of the Plant Pathology Department.

[2] The assistance of F. P. McWhorter and H. H. Millsap in the photographic work is gratefully acknowledged.

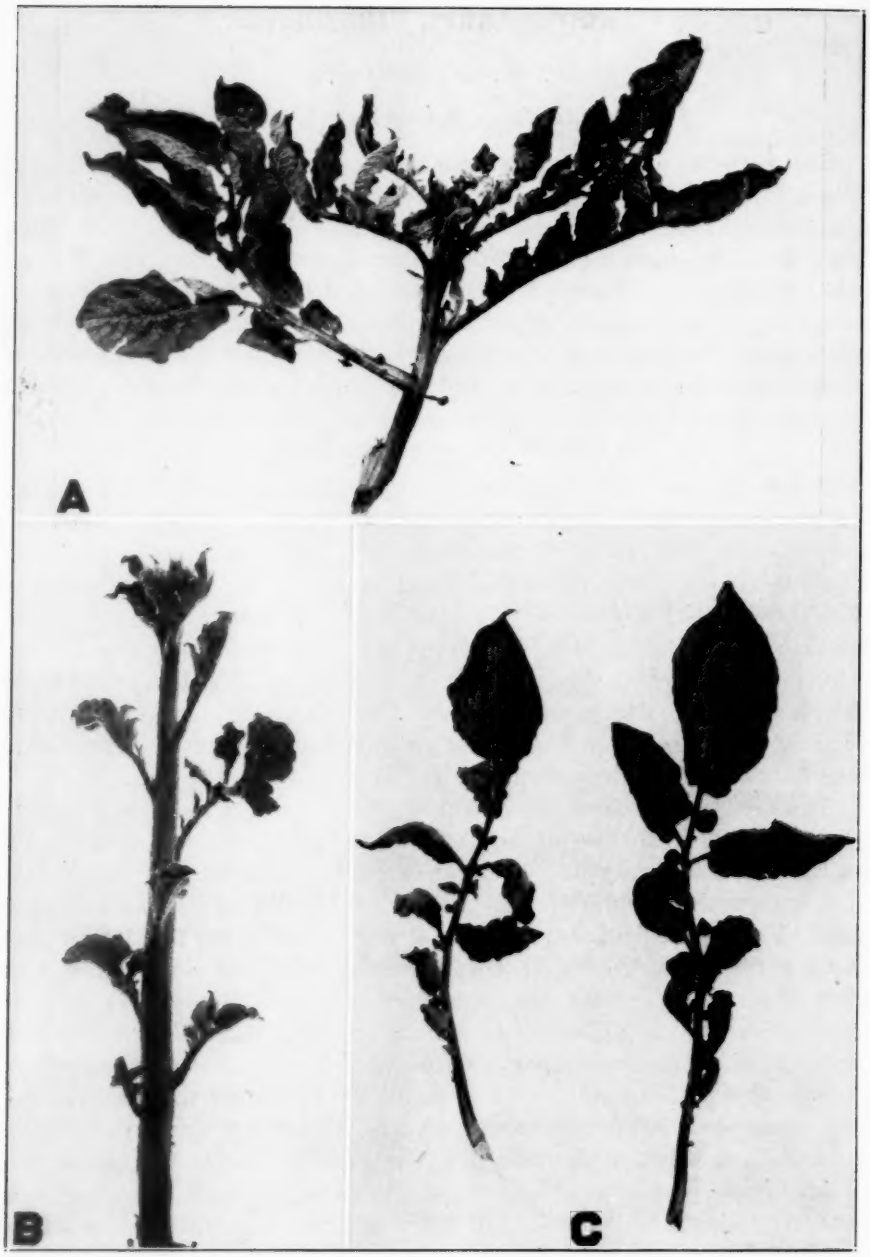

FIG. 1. A. Terminal growth of a field-grown Netted Gem potato plant affected with green dwarf. The symptoms are much the same for current-season and tuber-perpetuated green dwarf. B. A mature greenhouse-grown plant of Green Mountain from a tuber infected the previous year with green dwarf. C. Typical leaf symptoms of green dwarf on field-grown Netted Gems.

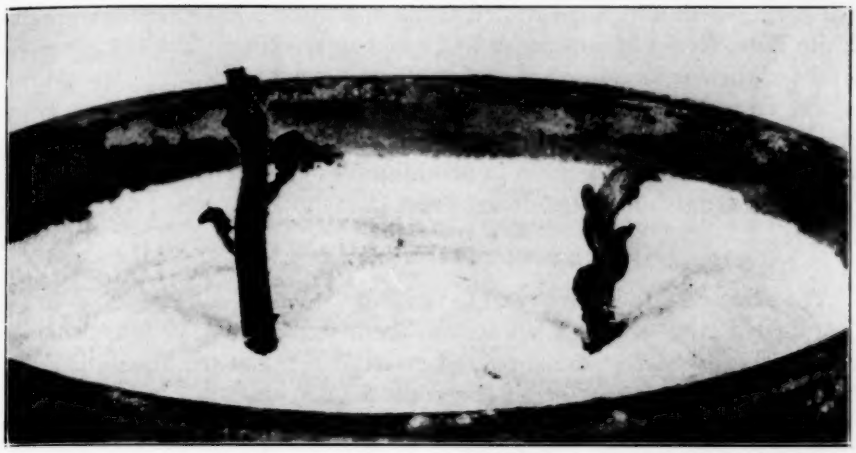

FIG. 2. Tuber-perpetuated symptoms of green dwarf. Photographed three weeks after emergence. All varieties studied emerge late and grow slowly after emergence.

Current season symptoms. The current season infection of green dwarf from natural spread causes very little injury to the plants. The symptoms are usually limited to the terminal growth of one or more lateral branches, and are very similar to the symptoms of the terminal growth of tuber-perpetuated green dwarf (Fig. 1, A). The growth stops or becomes very much dwarfed. The leaves are small and the leaflets are misshapened and cup upward (Fig. 1, C). The leaves of the growing point are clustered together or pinched and dwarfed.

No current season symptoms have been produced in the greenhouse by inoculation.

TRANSMISSION OF THE DISEASE

The disease has not been transmitted by the carborundum or the tuber core graft methods of inoculation. It is readily transmitted by grafting a portion of diseased stem onto a healthy plant. Green dwarf has been trans-

TABLE 1.—*Results of inoculating potato plants with the green-dwarf virus*

Variety	Method of inoculation	No. of plants inoculated[a]	No. of plants with green dwarf
Netted Gems	Carborundum	10	0
White Rose	do	5	0
Burbank	do	5	0
Netted Gems	Core graft	17	0
Netted Gems	Side graft	8	7
White Rose	do	9	2
Burbank	do	3	2
Green Mountain	do	4	4
X-immune seedling	do	5	1

[a] A similar number of check plants were grown for each inoculation. Tubers were saved from these plants and were again used as checks for tuber perpetuated symptoms. None of the checks developed green dwarf.

mitted by grafting infected Netted Gems to healthy Netted Gems, Burbank, White Rose, Green Mountain, and X-immune seedling. Table 1 gives the details of these inoculations.

The virus apparently moves slowly through the plant because tubers taken from plants with current season symptoms may not all produce green dwarf. Likewise, individual tubers cut in several pieces and planted as a tuber unit may produce only one green-dwarf plant in the unit.

LOSSES FROM THE DISEASE

The disease has not developed to the point where it causes serious losses. Most fields have less than 1 per cent of the disease. In some areas there is considerable current season spread of green dwarf, and the disease is a constant worry to growers of certified seed in these areas.

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PHYTOPATHOLOGICAL NOTES

Transmission of Peach Wart by Graft Inoculations with Affected Fruit Tissue.—On August 24, 1942, three J. H. Hale and three Elberta peach trees in the plots at Moscow, Idaho, were inoculated with fruit tissue affected with peach wart. Wedges of warty tissue approximately 35 mm. long, 3 mm. thick, and 4 or 5 mm. wide at the large end were placed in T cuts in the bark of the trunks of young peach trees. Three pieces of tissue were set on each tree and held in place with rubber budding strips. The external surface of the fruit was placed outermost and since the warty tissue was either very tough and leathery or woody there was very close contact between the wedges and the stock under the rubber band pressure. Observation showed that in several cases the peach fruit tissue retained its normal appearance and seemed alive for at least a month. Cochran and Rue[1] secured transmission of peach mosaic by a similar grafting technique using leaf, fruit, bark, and wood tissue. They state that in the case of leaf tissue it appeared to have united with the wound callus.

In August, 1945, examination showed that transmission had occurred in all cases inoculated by the fruit tissue method. No control inoculations were made with tissue wedges of healthy fruit but the trees inoculated by this technique or by bud inoculation were the only ones showing warty fruit in the plots which contained several hundred trees. The virus causing peach wart is known to spread in an orchard[2] but even in older plantings the spread is slow. The experience with peach wart and the experimental evidence presented indicate that the presence of the disease on these trees was not due to contamination but to actual transmission of the virus. Fruit symptoms were typical and severe.[2] It is not known whether tissue union occurred; according to Dorsey and McMunn[3] it is generally agreed that in nearly mature peach fruits there is no meristem present in flesh tissue. There remains the possibility, however, that in peach fruits affected by wart, some meristematic tissue may exist long after it has disappeared in normal fruits. Transmission by this fruit tissue method and variations of it offer a useful technique for studying properties of the peach wart, mosaic, and perhaps other viruses.—EARLE C. BLODGETT, Formerly Assoc. Plant Pathologist, Idaho Agricultural Experiment Station, Moscow, Idaho.

Stubborn Disease of Citrus, a Virosis.—At the time the history and the description of the stubborn disease on navel oranges was first reported,[1] it was suspected that it might be a virus disease rather than a genetic variation. The experimental evidence developed since that time together with further

[1] Cochran, L. C., and John L. Rue. Some host-tissue relationships of the peach mosaic virus. (Abstr.) Phytopath. **34**: 934. 1944.

[2] Blodgett, Earle C. Peach wart. Phytopath. **33**: 21–32. 1943.

[3] Dorsey, M. J., and R. L. McMunn. Tree-conditioning the peach crop. Ill. Agr. Exp. Sta. Bul. 507. p. 353–357. 1944.

[1] Faweett, H. S., J. C. Perry, and J. C. Johnston. The stubborn disease of Citrus. California Citrograph **29**: 146, 147. 1944.

observations has led to the definite conclusion that stubborn disease on navel orange trees is of virus origin.

The symptoms on navel oranges when pronounced consist of a brush-like growth of twigs, due to abnormal branching from multiple buds. The small branches often bend down over most of their length and turn up at the outer ends. The leaves tend to be broader and shorter and bend upward more on each side of the midrib than those of healthy trees. The foliage, especially on the south side, usually consists of an untimely autumn growth which becomes somewhat chlorotic. Some of the fruit develops abnormally into forms that resemble an acorn shape[1, 2] with the rind of the stem half or portions of it remaining normal in thickness while the rest of the rind decreases abruptly in thickness with the thinnest portion near the stylar end. In severely affected fruit the pulp below the thin portions of the rind has a sour or bitter taste and disagreeable odor. Trees tend to become unproductive and are often mistaken for the so-called "Australian type" of navel.

The most definite experimental evidence was obtained by budding sweet orange seedlings with buds from trees with stubborn disease (March, 1939) and topworking the resulting trees with buds from a healthy navel orange tree (May, 1943).[1] At the present time (March, 1946) the growth from the originally healthy navel buds has not only developed the typical branch and leaf symptoms, but some of the fruits have the "acorn" symptoms.[1, 2]

Healthy buds from this same source placed on trees at the same time, not showing stubborn symptoms, grew out and produced normal branches, leaves, and fruit.

The symptoms on the diseased trees developed slowly. This experiment corroborates many previous observations by propagators and growers that trees with these symptoms, when top worked with carefully selected healthy navel orange buds or scions, again had the same disease symptoms after developing a new top.

There has not been time as yet to determine definitely whether or not the so-called "crazy top" and "acorn" or "pink nose"[3] of grapefruit is the same disease although the symptoms are similar. Transmission experiments on grapefruit and other varieties were begun in 1944.

To summarize the results: Typical symptoms have been induced in tops of navel orange trees grown from healthy buds placed in diseased trees. Tops grown from healthy buds from the same source placed in healthy trees remained healthy.

The virus causing the stubborn disease may be designated and described as follows:

Citrivir pertinaciae[4] (pertinaciae, genetive of pertinacia = pertaining to stubbornness), the virus causing stubborn disease of citrus. Induces on

[2] Haas, A. R. C., L. J. Klotz, and J. C. Johnston. Acorn disease in oranges. California Citrograph 29: 148, 168–169. 1944.

[3] Burgess, P. S. Agricultural Chemistry and Soils: Boron. Arizona Agricultural Experiment Station Rept. 52: 7. 1942.

[4] Fawcett, H. S. Citrus viruses. Phytopath. 31: 356–357. 1941.

navel orange trees multiple buds and abnormal branchings resulting in a brush-like growth. Induces broader and shorter leaves than normal and untimely, somewhat chlorotic, growth in the autumn. Causes some of the fruit to take on an "acorn" shape in which the rind at the stem end is normal, becoming abruptly thinner and smoother over the remainder of the surface. Mature fruits tend to be sour and bitter at the navel end.—H. S. FAWCETT, Citrus Experiment Station, Riverside, California.

Experiments on the Overwintering in the Soil of Bacteria Causing Leaf and Pod Spots of Snap and Lima Beans.—The overwintering in nonsterile soil of bacterial pathogens of snap and lima beans (*Phaseolus vulgaris* and *P. lunatus* var. *macrocarpus* (Benth.) Van Eselt.) is still an open question, although in the case of *Xanthomonas phaseoli* (E. F. Sm.) Dowson there is good circumstantial field evidence for survival through the winter in bean plants thrown on compost heaps. The writer has made the following experiments:

Idaho-grown snap bean seed of the Bountiful variety was planted in May, June, and July at the Arlington Experimental Farm in Virginia, in a plot where snap beans heavily infected with *Pseudomonas medicaginis* var. *phaseolicola* (Burkh.) Stapp and Kotte had been plowed under the previous autumn. No infection ensued.

The soil hold-over of *Pseudomonas medicaginis* var. *phaseolicola* and *Xanthomonas phaseoli* was tested by burying in pots of Arlington Farm soil lima bean leaves and pods with fresh autumn infections of these organisms. The pots were sunk in the ground out of doors. The following spring Full Measure snap bean plants from Idaho seed were grown therein and kept well watered, but no signs of infection appeared on them.

A similar experiment on the Eastern Shore of Maryland likewise produced negative results. In this case the buried lima bean (King of the Garden variety) leaves bore fresh autumn infections of *Pseudomonas syringae* van Hall and the varieties planted in this soil the following spring were King of the Garden lima bean and Idaho-grown Bountiful snap bean.

Inoculation experiments to test further the overwintering of bean bacterial pathogens were made as follows:

Lima bean leaves with autumn infections of *Pseudomonas syringae* had been exposed to weathering from October 22 till June 7, but had not been in contact with the soil. On the latter date they were crushed to powder and dusted on sprayed pricked seedlings of Ideal pole lima in a damp chamber. The plants were sprayed again after dusting and kept 3 days in the damp chamber. No *Pseudomonas syringae* appeared on any of the 19 plants, but *Xanthomonas phaseoli* was isolated from water-soaked spots on inoculated leaves of 3 of them. The check plants had no sign of infection. Had the *Xanthomonas phaseoli* been associated with *Ps. syringae* in the lesions on the weathered leaves? *X. phaseoli* has been found now and then, in small numbers, in lesions caused by various other bean bacterial pathogens.

The preceding experiment was repeated a week later, but with a water suspension of the powdered dried leaves. No infection resulted.

Soil in which lima bean leaves with fresh autumn infections of *Pseudomonas medicaginis* var. *phaseolicola* had been buried out of doors on October 22, was used as inoculum on June 7. It was applied to the leaves of Ideal pole lima seedlings in a damp chamber, after which the plants were sprayed and pricked. No infection resulted.—FLORENCE HEDGES, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, Plant Industry Station, Beltsville, Maryland.

REPORT OF THE 1946 ANNUAL MEETING OF THE NEW ENGLAND DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

In order to coincide with the New England–New York Spray Specialists' Conference, the 1946 annual meeting of the New England Division was held on December 4, 5, 6, 1945. Members of A.P.S. in New York, New Jersey, and Pennsylvania were invited to participate in these meetings. Of the 75 persons who registered, approximately half were from the invited states. In addition to the 14 papers presented, 3 symposia were conducted: Potato Diseases, Seed Treatments, and Teaching Plant Pathology and Trends in Research.

Officers of the New England Division for 1946 are: President, Frank L. Howard, Rhode Island State College, Kingston; Vice President, P. J. Anderson, Connecticut Experiment Station, Windsor; Secretary-Treasurer, Thomas Sproston, Jr., Massachusetts State College, Amherst; Councilor, M. C. Richards, University of New Hampshire, Durham.

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

The Fungi of Pole-rot of Tobacco. ANDERSON, P. J. A considerable number of species of bacteria and fungi, *Alternaria, Macrosporium, Fusarium, Penicillium, Aspergillus, Cladosporium,* and others, are readily isolated from pole-rot damaged leaves of tobacco in the curing shed. When leaves are inoculated these species are found to be either nonpathogenic or so weakly pathogenic that they could not be responsible for destructive epidemics. Two other fungi, *Sclerotinia sclerotiorum* and *Botrytis cinerea,* rarely isolated from old lesions, are always isolated from the very young lesions, are highly pathogenic on tobacco leaves, and are responsible for the severe epidemics in New England. They are most active on leaves that are still green. Later these two fungi are destroyed by the other organisms mentioned. Pole-rot starts on tobacco while it is still standing in the field.

A Laboratory Assay for Stability of Organic Fungicide Residues. BARRATT, R. W. Laboratory assays have been designed to measure the following factors causing failure of fungicides in the field: tenacity, previous material in the spray tank or additives, decomposition during application, and decomposition of the spray residue from ultraviolet light, leaf exudates, atmospheric gases, rain water, and soil on leaves. The rate of decomposition is an important statistic. Essentially, the technique involves the spraying of coated glass slides in a dosage series employing a dose ratio of 2 or $\sqrt{2}$. Replications of each concentration are sprayed, one set being assayed immediately for fungicidal potency. The remaining slides are stored at a constant temperature in a closed chamber having the environment being studied. By assaying slides stored for different periods of time, the LD90 in terms of micrograms per sq. in. can be determined. From the shape and position of the curves resulting from charting the logarithm of the LD90 against the time of storage in days, the relative rates of decomposition of the fungicides can be determined. By using this technique the rate of decomposition of disodium ethylene bis dithiocarbamate in the presence of water and oxygen has been studied and can be slowed down by the addition of zinc sulphate and lime.

Control of Damping-off by a Delay in First Watering After Seeding. DORAN, W. L. When seeds were sowed in soils infested with *Pythium,* the soils having a moisture content of not more than 30 per cent of the water-holding capacity at time of seeding, damping-off, especially pre-emergence damping-off, was much less severe if soil was not watered for the first time until several days after seeding. Numbers of plants which lived in such soil first watered four or five days after seeding were, as compared with numbers which lived in soil watered immediately after seeding, increased by the following percentages: tomato, 39 per cent; eggplant, 27 per cent; pepper, 37 per cent; onion, 96 per cent; lettuce, 89 per cent; beet, 133 per cent; and cabbage, 124 per cent. Stands were not improved by

a delay in first watering if the soil at time of seeding had a much higher moisture content. Stands were usually more improved by a delay of four to five days before the first watering than by a shorter interval. They were usually as much improved by waiting four to five days as by waiting longer and any further delay may be undesirable because of a corresponding delay in emergence. The method is not suggested as a substitute for soil or seed treatment with fungicides but, lacking them, it is a worthwhile cultural practice.

Stimulation and Retardation of Germination of Some Vegetable Seeds Resulting from Treatment with Protective Fungicides. FOSTER, A. A. Copper fungicides injured cabbage, cucumber, and pea seed and stimulated beet, eggplant, pepper, and spinach seed germinated in Petri plates or steamed soil. Nitroprusside tests showed that copper-sensitive seeds contained the sulfhydryl radical while others did not. Copper treatment reduced or eliminated sulfhydryl groups in seeds. Addition of the amino acid, cysteine, which contains the sulfhydryl groups, resuscitated pea seeds that would not germinate following copper treatment. Measurements of oxygen uptake by ground pea and spinach seed showed that pea respiration was retarded by copper but that spinach respiration was not. Pea embryos from treated seed had lower fresh and dry weights than the check. The specificity is interpreted as evidence that the respiratory system of cabbage, cucumber, and pea is catalyzed by enzymes requiring the sulfhydryl group while the respiration of beet, eggplant, pepper, and spinach is catalyzed by an enzyme requiring copper. Effects of chloranil and tetramethyl-thiuram-disulphide could be interpreted similarly.

Distinguishing Permeation from Toxicity of Fungicides. HORSFALL, J. G., R. W. BARRATT, and A. GRIES. Usually, toxicity data do not distinguish between permeation of the chemical into a cell from damage to its contents. Sodium sulphate and other monovalent electrolytes known to increase permeability of cells have been found to increase the potency of disodium ethylene bis dithiocarbamate and *o*-quinone dioxime to *Macrosporium sarcinaeforme*. That this is due to increased permeability and not to increased toxicity is indicated by the fact that the effect can be counteracted by calcium and other divalent electrolytes which are known to reduce the permeability effects of sodium salts. If the toxicant is held constant and sodium sulphate is increased, potency waxes and wanes periodically, but the wavy curve can be rectified by varying concomitantly the amount of calcium. Assuming that the effect is due to variations in permeation through a colloidal gel, in this case the cell wall of the spore, the periodic characteristic resembles the phenomenon in Liesegang rings. Zinc sulphate can synergize disodium ethylene bis dithiocarbamate by liberating sodium sulphate into the medium as the zinc salt is being formed. The toxicity rises with addition of zinc sulphate until all the Na has been replaced, despite the fact that the zinc salt is less potent than the sodium salt. Apparently, the increased permeation more than counterbalances the reduced toxicity of the zinc salt. Calcium in the mixture, however, antidotes much of the effect.

Interactions of Concentration, Pressure, Time, and Orifice in Spraying. HORSFALL, J. G., NEELY TURNER, and A. D. MCDONNELL. The interactions of the factors in spraying were studied by applying variable amounts of material per acre. As the amount of Bordeaux mixture on potatoes or calcium arsenate on beans was increased, the rate of deposit increase was in the following order: concentration $>$ time $>$ pressure $>$ orifice diameter. Deposit increased more rapidly with short than with long spray time, with low than with high pressure, and with small than with large orifices. Using a balanced triangular design with concentration constant, calcium arsenate deposited best on potatoes with high pressure, short time, and small orifices. Deposits, however, may have different tenacity and coverage as a function of technique. The bigger the deposit of calcium arsenate, the faster it washed off in the rain. Applications with short spray times, therefore, washed more rapidly than those from long spray times and this tended to nullify the advantage in original deposition. An attempted assay of coverage was made with the log-probit dosage-response curve using Bordeaux mixture and leafburn on potato. The laboratory conclusion that good coverage should be evinced by a steep slope was not confirmed because of the strong concave curvature which presumably resulted from the fact that the big deposits washed more rapidly than the smaller.

Dutch Elm Disease Studies in Massachusetts During 1945. MCKENZIE, M. A. As of December 1, *Ceratostomella ulmi* (Schwarz) Buisman, the causal fungus of the Dutch elm disease, was isolated from 31 additional trees or wood samples during 1945 in Massachusetts. A total of 74 confirmations by culture for the fungus have been made since 1941: Berkshire County 54, Hampden County 18, Hampshire County (first report) 2. In all locations where the fungus was found during the year detailed surveys were conducted and related beetle-infested bark was burned by property owners, town tree wardens, city foresters, State departments, public utilities, and other cooperating agencies and indi-

viduals. As supplements to this control effort, suggestions were furnished tree owners concerning the general health of elms, spray programs for the control of leaf-feeding insects and particularly for the reduction to a minimum of the elm bark beetle population. Accordingly, the record of attempted control is indicated briefly. In general a few diseased trees have been found in succeeding years after the first afflicted tree was reported for a town, but increase of the disease has not been extensive. In the city of Westfield, the first diseased tree was found in 1942; a second tree was found affected in 1945. In the town of Alford, the first diseased tree was found in 1941; 2 additional ones were found in 1944. In the town of Richmond, 7 trees were confirmed for disease in 1945; apparently some build-up of the fungus occurred there before any diseased trees were found. In urban areas the amount of activity of the citizens is believed to be an important factor in disease increase, while in rural areas storm damage sometimes suddenly creates abundant material suitable for breeding by bark beetles. The increased use of oil as fuel in the postwar period may be an aid to disease control, if storage of elm as fuelwood is thereby reduced.

Apple Leaf Structure in Relation to Penetration by Spray Solutions. PALMITER, D. H., E. A. ROBERTS, and M. D. SOUTHWICK. Spray solutions containing minor elements, nitrogen, "hormones," and organic fungicides have been applied as foliage sprays to apple trees to reduce nutritional deficiencies, prevent fruit drop, or increase resistance to disease. The fact that such applications have given results showed that they had penetrated the leaves and raised the question of how penetration occurred. Standard microchemical technique for differentiating between cutin, cellulose, and pectic substance showed the avenue by which water-soluble materials might pass through the cuticle and surround the epidermal and interior cells. The apple leaf cuticle was not a solid mass of cutin but a laminated tissue composed of discontinuous layers of cutin, cellulose, and pectic materials. The stains showed a continuous avenue of pectinacious substance from the exterior of the cuticle to the epidermal cells and vein extensions. The penetration of apple leaves was demonstrated by placing cut terminals in a 0.5 per cent solution of ferric sulphate and spraying the leaves with a 0.3 per cent solution of sodium dimethyl dithio carbamate. As the sulphate, taken up by the stem, reacted with the carbamate, taken through the cuticle, a black precipitate formed from the cuticle along the veins and into the stem. It was most dense in areas of greatest pectic content.

Effect of Inorganic Fertilizers on Defoliation of New Hampshire Victor Tomatoes by Alternaria solani. RICHARDS, M. C., and R. C. JONES. In earlier investigations, tomato varieties having a heavy fruit to leaf ratio defoliated earlier than those with a high leaf to fruit ratio. The leaves appeared to become susceptible to attack by the pathogen as the nutrients were drained from the leaves by the rapidly developing fruits. In 1944 and again in 1945 attempts were made to supply the roots of the test plants with an excess of N, P, and K so that a higher level of these nutrients could be maintained in the leaves during the fruiting period. The New Hampshire Victor tomato variety was used, as it fruits abundantly and defoliates severely. Inorganic nutrients of N, P, and K were added to the soil at the base of each plant, about 6 inches deep, when the plants were set in the field from 3-inch pots. The tests were arranged in randomized blocks with five replications per treatment. In one block ammonium nitrate, calcium cyanide, ammonium sulphate, sodium nitrate, and Uramon (urea) were used as N sources and applied at the rate of 100 pounds of N per acre plus P_2O_5, 75 pounds, and K_2O, 45 pounds per acre. In other tests the N–P–K ratios were varied from 0–2–1.75 to 4–2–1.25, the nitrogen varying from 0 to 17, 35, 70, and 140 pounds per acre, with P and K remaining as given above. In further tests the total pounds of N, P, K per acre were varied. In general, there was an increase in the total fruits per plant with increases in nutrients applied. As a result, the high fruit to leaf ratio was maintained, and defoliation was as heavy on these as on the checks. Although abundant soil nutrients were available, a limited number of tissue tests did not reveal increases for N, P, or K in the leaf petioles.

Control of Alternaria Blight on Tomatoes with Fungicides. RICHARDS, M. C., and R. C. JONES. Six applications for each of eighteen fungicide treatments were made during the 1945 season on New Hampshire Victor tomatoes to control *Alternaria solani.* Single hill units with ten replicates for each treatment used were arranged in a randomized block. The concentrations of the materials used and their effect on control of defoliation were as follows: Dithane (disodium ethylene bisdithiocarbamate) ($\frac{1}{2}$–100 + $ZnSO_4$ and lime**), Puratized (phenyl mercuri triethanol ammonium lactate) (1–20,000**), Bordeaux (3–3–50**), Zerlate (zinc dimethyldithiocarbamate) (2–100**), Copper oxychloride sulphate (6–100**), Phygon (2,3-dichloro-1,4-naphthoquinone) (1–100**), Fermate Dust (ferric dimethyldithiocarbamtae) (10–90*), Tribasic $CuSO_4$ (3–100*), Spergon (tetrachloro-para-benzoquinone) (1–100*), Fermate + DDT (2–0.6–100), Fermate (2–

100), Fermate (1–100), Fermate Dust + DDT (10–3–87), Bismuth subsalicylate (1½–100), Fermate (1–100) + sticker, Q15 (lauryl isoquinolinium bromide) (½–100), No Fungicide. Average weights in pounds of marketable fruit per plant for each treatment were: Zerlate (8.20**), Puratized (7.93**), Phygon (7.70**), Fermate 2–100 (7.47**), Tribasic $CuSO_4$ (7.34**), Fermate 1–100 (7.09*), Bismuth subsalicylate (6.30*), Fermate 1–100 + sticker (6.00*), Dithane (6.00*), Fermate Dust (6.00*), Fermate + DDT (5.80), C.O.C.S. (5.67), Bordeaux (5.50), Fermate Dust + DDT (5.30), No Fungicide (5.00), Spergon (4.80), Q15 (3.98). None of the fungicides prevented eventual complete defoliation by *Alternaria*. Definite injury to foliage was noted with Dithane, and stunting of the plants was indicated with Bordeaux and Copper oxychloride sulphate.

** Highly significant (99: 1).

* Significant (19: 1), better than "No Fungicide."

The Incidence of Common Scab on Green Mountain Potatoes in Soils at Different pH Levels. STEINMETZ, F. H. A field which had been in sod for a number of years was fall plowed and laid out into plots to which agricultural lime and finely divided sulphur were applied in order to establish plots with calculated pH levels as follows: 4.5, 5.0, 5.5, 6.0, 6.5. The 4.5 pH series received 2000 pounds of sulphur per acre, the 5.0 pH series received 1000 pounds. The 5.5 pH series was not treated because the soil approximated this pH level. The 6.0 pH series received 2000 pounds of calcium carbonate per acre, while the 6.5 pH series received 4000 pounds. One series of these treated plots received 600 pounds of sulphur per acre. The amendments were applied only once, in the spring before planting the initial potato crop. The potato crop failed the first year on the plots receiving 2000 pounds of sulphur and was suppressed on the plots which received 1000 pounds. After the second potato crop the suppressing effect of sulphur was not marked. The plots which received the 600-pound rate of sulphur produced good yields of scab-free potatoes each year. During the first crop year none of the plots produced scabby potatoes. After 10 years, the increase of scab was in direct relation to the increase in pH level above 5.5. At present the plots with the original pH levels adjusted to 6.0 and 6.5 produce nonmarketable scabby potatoes.

Soil Applications of Oxyquinolin Benzoate for the Control of Foliage Wilting in Elms Caused by Graphium ulmi. STODDARD, E. M. Elm trees, 1.5 inches D.B.H., were treated in lots of 10 trees each with an aqueous solution of oxyquinolin benzoate in concentrations of 0.1, 0.05, 0.025, and 0.0125 per cent at the rate of 10 gallons per tree, applied to the soil. Each concentration was applied as a single application of 10 gallons and 5 applications of 2 gallons each on alternate days for 10 days. Duplicate series of applications were made 10 days before and 10 days after inoculation of the trees with *Graphium ulmi*. A concentration of 0.1 per cent reduced the trees that wilted to 27.5 per cent as compared to 73.4 per cent on the checks. Plots treated with this concentration uniformly had fewest trees wilting and the least wilting per tree, irrespective of manner or time of treatment. The plots treated with a single dose at all concentrations had more trees wilting and less wilting per tree than plots treated with multiple doses. There was no difference between plots treated before and after inoculation, either in number of trees wilting or the amount of wilting per tree. From the fact that *Graphium ulmi* was isolated from an approximately equal number of trees in all the treatments and the checks, it is suggested that the effect of the oxyquinolin benzoate was due to antidoting of the fungus toxin causing the wilting and was not due to fungicidal action.

Glyoxalidine Derivatives as Foliage Fungicides: Laboratory Studies. WELLMAN, R. H., and S. E. A. MCCALLAN. The glyoxalidine or imidazoline nucleus is: HN–CH=N–CH_2–CH_2. Sixty-five glyoxalidines containing substituents in the 1- or 2-positions were examined. In laboratory slide-germination tests, maximum fungistatic action is achieved with glyoxalidines having a straight chain substituent containing 13 to 17 carbon atoms in the 2- position. Substituents in the 1- position such as hydroxyethyl, aminoethyl, or butyl do not markedly affect fungistatic action. The various glyoxalidines are removed from solution by spores (or charcoal) in amounts proportional to their fungistatic action. Their action is fungistatic and not fungicidal. Addition of oil or lead arsenate increases fungistatic action in laboratory tests. The 2-heptadecyl glyoxalidine is inherently tenacious in thin films on glass slides. In greenhouse experiments maximum phytotoxicity is reached with the 11 carbon atom side-chain in the 2- position. The ratio, highest concentration giving no plant injury over LD50, for 1-hydroxyethyl 2-undecyl glyoxalidine is 13.5; for 1-hydroxyethyl 2-heptadecyl glyoxalidine it is 1450. Side chain unsaturation increases phytotoxicity as does increasing length of chain in the 1- position. Quaternary-ammonium addition compounds were as fungistatic and were more phytotoxic and less water soluble than parent compounds. These materials were phytotoxic to toma-

toes at concentrations which would not control late blight, and they were moderately effective against snapdragon rust though not phytotoxic.

Glyoxalidine Derivatives as Foliage Fungicides: Field Studies. THURSTON, H. W., JR., and J. B. HARRY. The following compounds: 2-heptadecyl glyoxalidine (C.P.I. No. 341), 1-hydroxyethyl 2-heptadecyl glyoxalidine (C.P.I. No. 337) and 1-aminoethyl 2-heptadecyl glyoxalidine (C.P.I. No. 630) have been studied in the field for five years. Crops and diseases under observation included roses (black spot), potatoes (late blight), apples (scab and rust) and sour cherries (leaf spot). On roses, Nos. 337 and 341 were tested and gave only fair control of black spot. On potatoes, Nos. 337 and 341 were tested and failed to control late blight; the compounds probably were injurious; and potato yields were not equal to those from Bordeaux plots. On apples, all three of the glyoxalidines gave scab control equal to the standard lime-sulphur spray program. The glyoxalidines gave less injury, especially to the foliage, and satisfactory color and finish to the fruit. The 2-heptadecyl glyoxalidine was compatible with lead arsenate, nicotine sulphate, and summer oil on apples and also controlled cedar-apple rust better than sulphur although not so well as Fermate. Four years' consecutive trials in Pennsylvania showed 2-heptadecyl glyoxalidine (341) to be superior to other fungicides tested, including 2–8–100 Bordeaux, in controlling the defoliation of sour cherries (Montmorency) caused by leaf-spot. Although this resulted in somewhat duller color, there was no reduction in size of fruit such as that brought about by Bordeaux.

REPORT OF THE THIRD ANNUAL MEETING OF THE POTOMAC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The third annual meeting of the Potomac Division of The American Phytopathological Society was held February 19 and 20, 1946, at the Plant Industry Station, Beltsville, Maryland. Officers elected for 1946–47 were E. E. Clayton, President; V. F. Tapke, Vice-President; W. F. Jeffers, Secretary-Treasurer; and R. J. Haskell, Councilor.

ABSTRACTS OF PAPERS PRESENTED AT THE THIRD ANNUAL MEETING OF THE POTOMAC DIVISION

Soil Fumigation against the Golden Nematode. CHITWOOD, B. G. The product D–D appears to be the most economical and effective soil fumigant used against the golden nematode of potatoes. Hand application of D–D at 425, 850, and 1700 lbs. an acre gave fumigation efficacy of 0.999, 0.996, and 0.999, respectively, with odds of 99: 1 that treatments were better than 99, 98, and 99 per cent effective. Experimental plots were surrounded by clean fields. Hand application of D–D at 425, 675, 850, and 1275 lbs. an acre gave efficacy of 0.974, 0.982, 0.985, and 0.989, respectively, with 99: 1 odds that treatments were better than 94, 95, 96, and 96 per cent effective. Plots were surrounded by infested fields. Machine-applied D–D at varied dosages per acre on 2 half-acre subplots per treatment gave the following efficacies: 452 lbs.—0.998 and 0.994 (better than 97 per cent); 631 lbs.—0.974 and 0.983 (better than 93 per cent); 858 lbs.—0.928 and 0.986 (better than 83 per cent); 930 lbs.—0.987 and 0.716 (second plot a failure); 1213 lbs.—0.991 and 0.973 (better than 93 per cent). Total overall efficacy was 0.938, with 99: 1 odds that treatments were better than 86 per cent effective. There were 15,255 females observed in controls, 953 in treatments; of the latter, 693 were in one subplot. Mechanical failure might explain the single exceptional subplot.

Aerosol Treatments for the Control of Tobacco Blue-Mold Disease. CLAYTON, E. E. The benzyl salicylate–cottonseed oil blue-mold spray has been adapted to the aerosol type of treatment and used successfully 2 years. The concentrate used was benzyl salicylate 4 ounces and cottonseed oil 1 gallon. By one method cartridges were loaded with 40 per cent concentrate and 60 per cent Freon. By another method small high pressure sprayers were loaded with a mixture of 43 per cent concentrate and 57 per cent acetone. Air pressure in the sprayer was adjusted to about 110 pounds. The rate of application was controlled by regulation of the spraying time. Small plants received 6 minutes spraying per 100 square yards and large plants up to 14 minutes. Excellent blue-mold control was secured.

Tobacco Resistant to Root Knot and Nematode Root Rot. CLAYTON, E. E., and T. W. GRAHAM. Root knot (*Heterodera marioni*) and nematode root rot (*Pratylenchus sp.*) are common and serious tobacco diseases through the flue-cured region of the Southeast. Search for resistance yielded one collection, T.I. 706, from which genotypes were selected that were highly resistant to both root knot and nematode root rot. This resistance has been consistent under the most severe field conditions in widely scattered locations. The full resistance was recovered after a cross with susceptible tobacco and after 1st and 2nd back crosses. Studies of back-cross lines showed segregation in all F_2 lines, but some F_3 lines were homozygous for resistance. Homozygous F_4 and F_5 lines have been secured for the 2nd back cross that appeared slightly more resistant to both root knot and nematode root rot than the original selection, T.I. 706.

Strawberry Virus in Eastern United States. DEMAREE, J. B. Preliminary studies indicate the presence of a virus disease of the yellows type in strawberries in Eastern United States. Distribution and prevalence have not been fully determined. There is some vein-clearing as well as a chlorosis of a transitory character in some varieties, appearing (if at all) in spring and fall, but the most pronounced and constant symptom is dwarfing due to short and horizontal growth of the petioles. Some varieties appear to be symptomless carriers. Artificial transmission by the stolon-grafting method in the greenhouse has been used during the past 3 years with a few eastern named and nameless varieties. The variety Marshall has proved satisfactory as an indexing plant.

Rate of Spread of Blueberry Stunt in a North Carolina Field. DEMAREE, J. B. The rate of spread of stunt (a virus disease) of blueberries varies greatly in different sections

and in fields in the same section. Its natural rate of spread is moderate to rapid in New Jersey, very rapid in some fields in North Carolina, and low to nil in Massachusetts, Michigan, and New York. Rate of dissemination in fields is undoubtedly associated with the presence and abundance of a vector. A part of a rectangular blueberry field (consisting of 10,000 plants set in 1936) was surveyed for 4 consecutive years for rate of spread of the disease. In lieu of surveying the entire field, 3 widely separated 10-row blocks of 600 bushes each were selected for surveying and designated as blocks 1, 2, and 3. Block 1 was laid out across the field near one end, block 2 was a section across the middle of the field, and block 3 was across the opposite end. Rate of spread of the disease in the 3 blocks from 1942 to 1945, inclusive, as indicated by percentages of affected plants, was as follows: *Block 1*—1942, 9.0; 1943, 23.3; 1944, 31.7; and 1945, 47. *Block 2*—1942, 4.7; 1943, 13.7; 1944, 23.7; and 1945, 47. *Block 3*—1942, 0.7; 1943, 1.5; 1944, 3.3; and 1945, 7.8. A few infected bushes were known as early as 1940 in block 1. The disease spread rapidly in that end of the field, and now nearly 50 per cent of the plants over at least half of the field are affected. After 6 years it has also become well established at the opposite end, a distance of about 200 yards.

Occurrence of a Strain of Tobacco-Etch Virus on Sweet Peppers in the Field. DOOLITTLE, S. P. Sweet peppers in Maryland and New Jersey occasionally have a virus infection which mottles the young leaves with small ring markings. Older leaves may have large, necrotic rings and eventually drop. Fruits of such plants often have large, concentric ring patterns and drop before maturity. These symptoms are largely confined to strains of the variety California Wonder and seldom appear on World Beater. A strain of tobacco-etch virus has been present in such plants. Inoculations with this virus alone, or in combination with the ordinary tobacco-mosaic virus or the cucumber-mosaic virus, have not produced ring markings of the fruit or leaves, although etch virus alone has produced leaf mottling similar to that seen in the field. When peppers are inoculated with both the etch and the tobacco-mosaic viruses, however, the leaf-mottling resembles that of etch virus alone, but there is severe defoliation of the plants. Fruits yellow and shrivel and have small, raised areas that produce a pebbled appearance. Fruit symptoms appear on California Wonder but rarely on World Beater. A disease of this type has caused serious losses in the field. With the combined infection, the defoliation has been more rapid than that sometimes caused by tobacco mosaic alone and, while the latter virus occasionally causes some yellowing and shriveling of the fruit, the fruit symptoms caused by the combined infection have been more consistently severe and of a typical character.

High Resistance to Common Tobacco Mosaic in Certain Lines of Lycopersicon hirsutum. DOOLITTLE, S. P., W. S. PORTE, and F. S. BEECHER. The wild tomato species, *Lycopersicon hirsutum* Humb. and Bonpl., is very tolerant of both yellow and green strains of the common tobacco-mosaic virus. The majority of plants inoculated have no symptoms, but virus is present in such plants in varying and fairly high concentrations. In 1941, however, two plants without symptoms when inoculated with a yellow strain of the virus, were found to be virus-free when tested by inoculations on *Nicotiana glutinosa*. Further inoculations produced no infection and cuttings from the plants have been carried as clonal lines. Continued inoculations, chiefly with yellow strains of the virus, have produced no infection in any of the 38 cuttings of one line tested during the past 4 years. All plants were young and growing vigorously and often were inoculated more than once. In the second line, 5 of 42 cuttings have had a trace of virus after inoculation but inoculations of the remaining plants failed to cause infection. Owing to difficulty in securing seed from *L. hirsutum*, trials with seedling progenies of these lines have been limited, but 21 of 58 seedlings tested have had no infection. Crosses between these lines and varieties of the cultivated tomato have yielded tolerant individuals, but none have had the high resistance of the wild parents.

Factorial Studies on Dithane Plus Zinc Sulphate–Lime: the "Reaction Product" (Zinc Ethylene Bisdithiocarbamate). HEUBERGER, J. W. In 1943 the addition of zinc sulphate and lime to Dithane (disodium ethylene bisdithiocarbamate) increased the control of potato early blight from 5 to 90 per cent and increased yield from 154 to 220 bu. an acre. Factorial studies in 1944 on potato early blight showed that the addition of lime alone to Dithane did not increase control, that the addition of zinc sulphate alone markedly increased control, and that the addition of both zinc sulphate and lime was slightly less effective than adding zinc sulphate alone. This result suggested the preparation of the reaction product of Dithane–zinc sulphate, a white flocculate, as a dry powder. Such a preparation was made, at the writer's request, by the Rohm and Haas Company in 1945. The flocculate was filtered, dried, and brushed through a fine-mesh screen. Control of potato early blight in 1945 was: Check, 0 per cent; Dithane, 15; Dithane plus zinc sulphate, 87; Dithane plus lime, 15; Dithane plus zinc sulphate–lime,

80; Reaction Product powder, 87. Yields were, respectively: 76, 93, 156, 101, 157, and 178 bu. an acre; yield with Bordeaux was 135 bu. an acre. No injury was observed from the Reaction Product powder, which, chemically, is zinc ethylene bisdithiocarbamate.

Apparatus and Small Scale Field Plot Design for Evaluating Fungicides on Vegetables. HEUBERGER, J. W., and T. F. MANNS. The pathologist who deals with field screening of numerous potential fungicides is faced with two problems: (1) the most efficient apparatus and (2) the most economical plot design. Where 50 or more compounds are evaluated in replicated, randomized block designs it is obvious that commercial sprayers or dusters require too large a plot size; knapsack sprayers and dusters require too high a labor output. Apparatus and plot design used in Delaware is as follows: A 3½-gallon-per-minute pump, operated by a small air-cooled motor, is mounted on skids. This is transported in a two-wheel trailer of sufficient size to carry 500 ft. spray hose, 500 ft. garden hose, five 15-gallon barrels, spray booms, etc. Sprays are mixed in 15-gallon barrels; as one is sprayed out the other is prepared. Spraying is done with a hand boom having 2 nozzles on top and two at the sides. Plots are laid out in a long rectangle having one block on each side of a center driveway, and each block is divided, thus making 4 replicates. Replicate size is 20 plants for potatoes and 13 plants for tomatoes. The apparatus and plot design permit application per hour of 12 to 16 treatments on potatoes and 8 to 10 on tomatoes.

Investigations on the Possible Growth-Regulating Effect of Several Fungicides. JEFFERS, WALTER F. In the course of testing fungicides as dips for seed sweet potatoes it was observed that several materials caused a significant increase, over nontreated roots, in the number of sprouts produced, even when disease control was apparently not a factor. To test the possibility that some fungicides have a stimulatory effect on plant growth, several experiments on the application of such materials to bean plants were conducted. Spergon (tetrachloro parabenzoquinone) was the only material, which when applied either in lanolin or Carbowax 1500 to the stems of beans, consistently gave positive curvatures. This curvature was very slight as compared with that produced by indole acetic acid and 2-4-dichloro phenoxyacetic acid. Several fungicidal materials were toxic when applied in the above manner; 1 milligram being the amount applied. As bean plants were not entirely satisfactory for this study, pieces of sweet potato were dipped in Spergon and in water and incubated for 9 days at room temperature. Spergon stimulated root growth and cell proliferation.

Regional Differences in Resistance of Hevea Selections to South American Leaf Blight. LANGFORD, M. H. Hevea rubber seedlings and clones assembled from many parts of the world have been exposed to South American leaf blight (*Dothidella ulei*) in widely scattered tropical American nurseries during the past five years. Sensitivity of the fungus to weather conditions, together with regional variation in disease severity, emphasized the need of thorough resistance tests in more than one locality for reliable selection work. Groups of clones from several widely separated areas of the native habitat of *Hevea* have been grown under comparable exposure to blight in regional test plots in Costa Rica, Panama, Trinidad, Brazil, and Peru. Likewise, large populations of Hevea seedlings from jungle trees have been tested at the place of their origin and in other areas. Both clones and seedlings that have proved highly susceptible in some areas have been damaged slightly or not at all in certain other areas. Prolonged exposure, however, has brought many cases of increased disease severity as variants of the fungus appeared. Some clones have proved highly susceptible to practically all regional populations of the fungus, others have been susceptible to certain variants only, and still others—now recommended for commercial use—have been highly resistant in all areas.

Golden Nematode on Commercial Potatoes. MACHMER, J. H. Examination of graded tubers and accompanying soil from heavily infested fields has shown that such potatoes as prepared for shipping may contain up to 379,500 golden nematode cysts per carload. Attempts to eliminate cysts in adherent soil and attached to tubers, by a combination of dry brushing and washing, succeeded in removing 86 per cent of the cysts. Jet-washing tested in a preliminary way removed 90 per cent of the cysts. A higher percentage reduction in cysts by jet-washing might be obtained with improved technic.

What Is Net Necrosis of Potato? MANNS, T. F. Severe net necrosis has been observed many times in the Central and Eastern States by the writer during the past 35 years during seasons of much leafhopper injury. Pathologists in northern potato-seed-growing states associate net necrosis with leaf roll, though they state these symptoms are not in the final stages of leaf roll. Appel and Orton, W. A., stated that net necrosis was not a reliable symptom of leaf roll. Entomologists, as far as I know, have

never associated net necrosis with leaf-hopper injury. Recently Severin has shown that a hopper may produce necrosis in beet with no virus present. In 1945 in a compatibility test of DDT with other sprays, two varieties of potato, Cobbler and Sebago, were northern grown and certified; two varieties, Green Mountain and Pontiac, were Delaware grown and 50 per cent of tubers had severe net necrosis. An experiment included four sprays with (a) DDT, (b) DDT plus calcium arsenate, and (c) unsprayed. The yield from the DDT plot was double that from the unsprayed plot. No leaf roll developed in the potatoes affected with net necrosis. Fortunately no fungus disease complicated the picture. Leafhoppers reduced the yield 50 per cent and net necrosis developed rapidly on the unsprayed potatoes in storage. The evidence is that net necrosis is leafhopper injury.

The Effect of Fungicides and Growth Substances on Easter Lily Bulb Production in the Field. MCCLELLAN, W. D., and NEIL W. STUART. In August, 1944, 315 Easter lily bulbs (U.S.D.A. Clone 6, which is relatively resistant to Fusarium basal rot), averaging 8.6 inches in circumference, were scaled and the scales treated with talc, Arasan (tetramethyl thiuram disulfide), or Fermate (ferric dimethyl dithiocarbamate), alone or containing 0.1 or 0.02 per cent of naphthaleneacetic acid (NA). The scales were planted in a 3 by 3 factorial in 5-bulb units in 7 randomized blocks in the field at Beltsville, Maryland. The bulbs were from 3 sources: (1) forced in Haydite in subirrigated beds; (2) forced in soil in pots; and (3) grown continuously in the field. In August, 1945, the bulbs were harvested, graded, counted, and weighed. There were no significant differences in the amount of basal rot between treatments. However, the greatest amount of basal rot occurred in those bulbs having the Haydite history (1), less in those with the greenhouse pot history (2), and none in those with the field history (3). Numbers of bulbs produced per 100 scales were as follows: talc, 393; 1:1000 NA-talc, 309; 1:5000 NA-talc, 379; Arasan, 513; 1:1000 NA-Arasan, 538; 1:5000 NA-Arasan, 418; Fermate, 488; 1:1000 NA-Fermate, 515; 1:5000 NA-Fermate, 456. Differences between treatments were greatest with the Haydite-history bulbs (1) and least with the pot-history bulbs (2). In 1943 and in 1944 Easter lily bulbs were treated with fungicides and combinations of fungicides with growth substances (16 different treatments in 1943 and 17 in 1944), but when the bulbs were harvested no one treatment could be rated as superior to the untreated controls.

The Effect of Fungicide-Growth-Substance Combinations on Herbaceous Cuttings. MCCLELLAN, W. D., and NEIL W. STUART. In an attempt to reduce losses due to rot, with fungicides, cuttings of geranium, snapdragon, chrysanthemum, and carnation were set in sand after dipping in dry talc, Spergon (tetrachloro parabenzoquinone), Arasan (tetramethyl thiuram disulfide), and Phygon (2,3-dichloro-1,4-napthoquinone) alone or containing 0.1, 0.04 or 0.02 per cent of naphthaleneacetic acid, naphthalene acetamide, or indolebutyric acid. The three fungicides reduced the percentage of rooting in all species except geranium. Undiluted Arasan severely injured chrysanthemum but not carnation cuttings. Spergon, however, was not injurious to chrysanthemum but severely injured carnation cuttings. Inclusion of 0.1 per cent of the growth substances in the fungicides increased percentage of rooting in tetraploid snapdragons and in one variety of chrysanthemum cuttings. In general naphthaleneacetic acid was the most effective of the growth substances. In other tests, dilution of Arasan and Fermate with talc (dilutions containing 1 to 20 per cent fungicide) prevented reduction in percentage of rooting of chrysanthemum and geranium cuttings. Addition of growth substances to the talc-fungicide mixtures increased the percentage and amount of rooting. Undiluted Fermate, Spergon, and Arasan used on poinsettia cuttings produced rooting and vigor ratings of 177, 139, and 107, respectively, as compared with 156 for talc. There was an insufficient amount of cutting rot in any of the tests to permit any conclusions as to the fungicidal value of the materials.

Golden Nematode as a Quarantine Problem. MCCUBBIN, W. A. In 1944 a survey of potato fields in 19 northeastern States indicated that this potato root parasite is probably not long or widely established elsewhere than in Long Island. In 1945 intensive survey in all Long Island potato areas disclosed no infestation except in 5 additional farms in the infested area environs. A quarantine established by the State of New York in March, 1944, and revised February 15, 1946, controls outward movement of nursery stock, rooted plants, and topsoil, requires the cleaning of transported tools and implements, and restricts potato shipment to New York City or to chip and starch plants. A plan now under discussion proposes to prohibit potato and root crop production on infested land and give it a D-D treatment to minimize spread. Prospects for successfully dealing with this pest in the years to come are uncertain. The nema may have been introduced from abroad into other areas as yet undiscovered; early potato shipments from infested Long Island farms may already have carried this pest elsewhere; and, finally, no known method

of survey can detect small, incipient outbreaks except by chance, so that new centers may remain undiscovered until well developed.

Progress on Tropical American Rubber Planting Through Disease Control. RANDS, R. D. Since 1940 the U. S. Department of Agriculture has conducted cooperative research and furnished technical guidance to 13 tropical American Republics interested in rubber planting. Control of leaf blight (*Dothidella ulei*) by spraying, as developed by Langford, and by crown budding with resistant Ford selections has removed the main obstacle which in the past nullified all attempts to establish tropical American plantations. These effective and economical procedures have enabled immediate use of the highest yielding Oriental clones, all of which are very susceptible to leaf blight. Commercial planting has now emerged from the nursery stage in several of the countries, and to the end of 1944, more than 28,000 acres of high-yielding rubber had been established. Development of a permanent, self-sustaining, small-farm or single-family type of rubber production is emphasized. This has required adjustment of local farm credit, colonization and other programs of the cooperating governments, which together with their nationals have reported total expenditures through 1945 of about $3,844,000 on their rubber projects. While the planting of crown-budded Oriental clones has proceeded, a cooperative breeding program has furnished thousands of first and second generation hybrids, some of which have given indication of combining superior yield with blight resistance.

Effect of Seed Treatment on Soybeans Germinated at Four Temperatures. SHERWIN, HELEN S., C. L. LEFEBVRE, and R. W. LEUKEL. Seedling emergence of soybeans was significantly increased when the seed was treated with Arasan (tetramethyl thiuram disulfide), New Improved Ceresan (ethyl mercury phosphate), and Spergon (tetrachloro parabenzoquinone), and planted in soil maintained at 15°, 20°, and 25° C. At 30° C., however, there was no significant increase in emergence. The soil in all cases was adjusted to 55 per cent water holding capacity. In the 12 seed lots tested, greater improvement in emergence was obtained from the treatment of seed that was produced in Mississippi than from similar treatment of seed produced in Illinois. Seed lots having low germination seemed to be benefited more by Arasan and New Improved Ceresan than by Spergon.

The Present Status of Breeding for Disease Resistance in Oats. STANTON, T. R. The most important new disease-resistant varieties have been developed by crossing Richland on Victoria, resulting in Vicland, Tama, and similar varieties, grown on about 25,000,000 acres in 1945. Less important groups of disease-resistant winter oats have been developed by crossing Fulghum, Nortex, and Lee on Victoria and by crossing Fulton type early red spring oats and Victoria-Richland selections. New varieties developed from crosses on Bond with better resistance to crown and stem rusts, greater productiveness, higher test weight, and stiffer straw are being released. These include Clinton and Benton in Iowa, Indiana, and Illinois; Bonda and Mindo in Minnesota; and Eaton in Michigan. Forvic [Forward × (Victoria-Richland)] is a promising new disease-resistant variety in Wisconsin. Threatening new or uncommon races of smuts, rusts, and other diseases are becoming important, such as: Races of smut that are virulent on Victoria, Boone, Vicland, Letoria; race 45 and similar races of crown rust that infect Bond, Clinton, and Benton; races 8 and 10 of stem rust; *Helminthosporium* diseases on recently distributed varieties; and oat viruses in the Southeastern States. Evidently, much yet remains to be done if all these diseases are to be more completely controlled by breeding. (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture and State Agricultural Experiment Stations.)

Distribution, Host Range, Character, and Significance of the Golden Nematode as a Disease Agent. STEINER, G. The golden nematode, *Heterodera rostochiensis* Wollenweber, occurs in Great Britain (including Ireland and Jersey Island), Sweden, Denmark, some northern and northwestern Provinces of Germany, and Long Island, U.S.A. It was known as potato dab or potato sick soil in Yorkshire around 1903; Zimmermann in Mecklenburg first recognized a nematode as its cause (1913) and Wollenweber described it as a separate species (1923). It was first observed in Sweden in 1922; in the United States in 1941. White potato and tomato are the main hosts; certain solanaceous weeds, also *Atropa belladonna* L. and *Chenopodium album* L., have been reported attacked. Tests by Ellenby showed some 50 tuber-forming South American species of *Solanum* invaded but generally to a lesser degree than white potato. *H. rostochiensis* lives sedentary on roots and tubers, the female transforms into a cyst remaining in the soil up to 10 years and containing viable eggs up to 8 years. This longevity of the cysts and the fact that tubers of affected plants appear healthy make it a particularly dangerous pest, usually not recognized before large infestations have been built up. Crops have been reduced to less than seed. An attempt to eradicate it on Long Island is planned.

Seed Treatment of Castor Beans for the Control of Seedling Blight. STEVENSON, E. C. Seedling diseases of castor beans caused pre-emergence and post-emergence blight or die-back; however, many affected plants which were not killed developed normally after the first true leaves were formed. In such plots the plants were later in maturing and the yield was reduced, but the quality of the seed was not affected. Seed treatment tests were made in 1943, 1944, and 1945. In 1943 the use of Semesan (hydroxymercurichlorophenol), New Improved Semesan Jr. (ethyl mercury phosphate), Spergon (tetrachloro parabenzoquinone), Arasan (tetramethyl thiuramdisulfide), and Thiosan (tetramethyl thiuramdisulfide) resulted in significantly better emergence. In 1944 and 1945 Semesan and New Improved Semesan Jr. were dropped from the test because of the shortage of mercurial compounds at that time. Arasan, Fermate (ferric dimethyl dithiocarbamate), and Spergon were tested at Manhattan, Kansas, and Stillwater, Oklahoma, in 1944, and at Beltsville, Maryland, in 1944 and 1945. The use of Arasan improved emergence significantly at Manhattan, Kansas, but none of the treatments was effective at Stillwater, Oklahoma. (Very little disease was present in the Kansas-Oklahoma areas during the year of the test.) Emergence was improved significantly by all three treatments in the two-year test at Beltsville, Maryland. Arasan was more effective than either Spergon or Fermate, the latter two not differing from one another statistically. Plant vigor and seed yield were not affected by any of the treatments.

A Simple, Effective Method for Inoculating Barley Seed with Helminthosporium gramineum. TAPKE, V. F. It has been difficult to develop a satisfactory simple method for the artificial inoculation of barley with the stripe fungus, *H. gramineum.* The following new method is relatively easy to apply and has consistently resulted in high infections with only small reductions in seed germination: The stripe fungus is grown on a nutrient broth in half-liter Erlenmeyer flasks for 10 days at room temperature, the mycelial pads are then fragmented in a homogenizer and applied in aqueous suspension for 15 minutes to seed that has been thoroughly dried following a 6-hour soak in water at approximately 25° C. During the first minute of the soak, the vessel containing the seed and inoculum is vigorously shaken. Next the inoculated seed is drained and incubated on moist blotters at 7° to 10° C. for 4 days, then dried at room temperature and sown. Through this method up to 100 per cent striped plants have been obtained in susceptible winter barleys grown in the field.

Spread of the Dutch Elm Disease in Maryland. WALKER, E. A. The Dutch Elm disease (*Ceratostomella ulmi*) became a serious menace to elm trees in Frederick County, Maryland, during 1944–45. The first diseased tree in Maryland was found at Ft. McHenry, Baltimore, in 1933. An outbreak was checked at Brunswick, Frederick County, in 1935, and near Cumberland, Allegany County, in 1936. No outbreaks have occurred in Baltimore or Cumberland areas since 1941. A light outbreak was observed in 1943, on 8 trees in Frederick County. This increased to 29 diseased trees in 1944, and five cases were reported for Carroll County. The elm bark beetle (*Scolytus multistriatus*) population increased greatly in 1944, and by 1946, 261 confirmed trees were found in Frederick County; 5 in Carroll, and 1 in Washington County. The disease is now confined mostly to the Monocacy River Valley, and its feeder streams. During 1944–45 State Officials removed and destroyed 101 diseased elm trees in Frederick County, 7 in Carroll County, and one in Washington County; and 245 other bark-beetle infested, dead and dying elms in Frederick County; and 47 in Carroll County. Since 1933, 120 confirmed elm trees have been removed in Maryland. The wet 1945 season greatly reduced the elm bark beetle population in Maryland.

Field Control of Bean Rust with Sulfur. ZAUMEYER, W. J. During the 1945 season bean rust in Pinto and Great Northern bean acreages was well controlled in Colorado, Wyoming, and Montana with sulfur dust applied at the rate of 20 to 25 pounds per acre. In the bean-growing sections of these States environmental conditions were ideal for the spread and development of rust. Early in the season infection is usually sparse and the control at this time is relatively simple, thus preventing secondary spread. In the Greeley, Colorado, area one dusting applied by most growers in early July, before the plants covered the rows, practically eliminated the disease. In Wyoming only a small percentage of the fields were dusted. Fields dusted twice yielded on an average of 1600–1800 pounds of seed per acre while fields not dusted averaged from 800 to 1000 pounds. In the Bridger, Montana, section where rust was widely distributed, 9 fields (totaling 330 acres) dusted twice averaged 2000 pounds of clean seed per acre. Eight fields (comprising 164 acres) dusted once averaged 1400 pounds, and 18 fields not dusted (totaling 364 acres) averaged 1016 pounds per acre. The best-producing, twice-dusted field yielded 2369 pounds and the poorest of the nondusted fields yielded 380 pounds of seed per acre.

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